การเปลี่ยนแปลงของอินนูลินในหัวแก่นตะวัน (*Helianthus tuberosus* Linn.) ก่อนและหลังการเก็บเกี่ยว

นางสุกัญญา ไหมเครือแก้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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PRE - AND POST - HARVEST CHANGES OF INULIN IN JERUSALEM ARTICHOKE (*Helianthus tuberosus* Linn.) TUBERS

Sukanya Maicaurkaew

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology

Suranaree University of Technology

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PRE - AND POST - HARVEST CHANGES OF INULIN IN JERUSALEM ARTICHOKE (*Helianthus tuberosus* Linn.) TUBERS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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สุกัญญา ไหมเครือแก้ว : การเปลี่ยนแปลงของอินนูลินในหัวแก่นตะวัน (*Helianthus tuberosus* Linn.) ก่อนและหลังการเก็บเกี่ยว (PRE - AND POST - HARVEST CHANGES OF INULIN IN JERUSALEM ARTICHOKE (*Helianthus tuberosus* Linn.) TUBERS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สุเวทย์ นิงสานนท์, 108 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อที่จะศึกษาระยะเวลาการเก็บเกี่ยวที่เหมาะสมและเพื่อที่จะศึกษาผลกระทบของสภาวะในการเก็บรักษาที่มีต่อปริมาณอินนูลิน และกิจกรรมของ fructan:fructan 1-fructosyl transferase (1-FFT)

ในการศึกษาเวลาเก็บเกี่ยวหัวแก่นตะวันเก็บเกี่ยวหลังจากดอกออกได้ 30, 40, 50, 60 และ 70 วัน ผลการศึกษาพบว่าช่วงเวลาการเก็บเกี่ยวหัวแก่นตะวันมีผลต่อปริมาณของอินนูลิน และมีผลต่อ inulin profiles และกิจกรรม 1-FFT โดยเมื่อระยะเวลาการเก็บเกี่ยวเพิ่มขึ้นกิจกรรมของ 1-FFT มีค่าลดลง (P<0.05) และช่วงระยะเวลาการเก็บเกี่ยวหัวแก่นตะวันที่เหมาะสมที่สุดคือ 50 วันหลังดอกออก พบว่ามีปริมาณอินนูลินสูงสุด และมีอินนูลินที่มีสายยาวที่สุด และพบว่าหัวแก่นตะวันที่เก็บเกี่ยวหลังดอกออก 60 วันมีปริมาณอินนูลินต่อไร่สูงที่สุด

ผลของอุณหภูมิในการเก็บรักษาหัวแก่นตะวันพบว่าอุณหภูมิในการเก็บมีผลต่อปริมาณอินนูลินในหัวแก่นตะวันระหว่างการเก็บรักษาอินนูลินจะลดลงในขณะที่น้ำตาลฟรุกโตสและซูโครสจะเพิ่มขึ้นซึ่งมีความสัมพันธ์กับการเพิ่มขึ้นของกิจกรรม inulin hydrolase (lnH) (P<0.05) และการลดลงของกิจกรรม 1-FFT การเก็บรักษาที่อุณหภูมิต่ำมีผลทำให้ลดปริมาณการสูญเสียอินนูลินและมีผลต่อการลดลงของกิจกรรม 1-FFT น้อยกว่าที่อุณหภูมิห้อง (25 ^{o}C) การเก็บรักษาที่อุณหภูมิ -18 ^{o}C สามารถลดการสูญเสียอินนูลิน และทำให้ปริมาณน้ำตาลฟรุกโตส ซูโครส กลูโคส ไนโตส และกิจกรรม 1-FFT คงตัวมากกว่าการเก็บรักษาที่ 0, 5, 10, 15 และ 25 ^{o}C

คาร์บอนไดออกไซด์ที่ความเข้มข้นร้อยละ 0, 5, 10, 15 และ 20 มีผลทำให้ปริมาณอินนูลิน น้ำตาลฟรุกโตส ซูโครส และกิจกรรม inulin hydrolase ในหัวแก่นตะวันมีความแตกต่างกัน (P<0.05) หลังการเก็บรักษา 30 วัน การสลายตัวของอินนูลินส่งผลให้ปริมาณอินนูลินลดลง และปริมาณฟรุกโตสและซูโครสเพิ่มขึ้น ซึ่งมีความสัมพันธ์กับการเพิ่มขึ้นของกิจกรรม lnH และการลดลงของกิจกรรม 1-FFT การเก็บรักษาด้วย CO_2 20% สามารถลดการสูญเสียปริมาณอินนูลิน และชะลอกิจกรรมของ lnH ได้ดีที่สุด เมื่อเปรียบเทียบกับที่ความเข้มข้น 0, 5, 10, และ 15 %

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2556 ลายมือชื่ออาจารย์ที่ปรึกษา _____

SUKANYA MAICAURKAEW : PRE - AND POST - HARVEST CHANGES OF INULIN IN JERUSALEM ARTICHOKE (*Helianthus tuberosus* Linn.) TUBERS. THESIS ADVISOR : ASST. PROF. SUWAYD NINGSANOND, Ph.D.,108 PP.

PRE HARVEST/POST HARVEST/INULIN/JERUSALEM ARTICHOKE TUBERS/ *HELIANTHUS TUBEROSUS*/FRUCTAN:FRUCTAN 1- FRUCTOSYL TRANSFERASE (1-FFT)/INULIN HYDROLASE (lnH)

The objectives of this study were to investigate the suitable harvest time and to determine the effects of the storage conditions on inulin profiles, inulin content, and the activity of fructan:fructan 1-fructosyl transferase (1-FFT).

Jerusalem artichoke tubers were harvested at 30, 40, 50, 60, and 70 days after flowering. Tuber maturity contributed to changes in inulin content, inulin profiles, and 1-FFT-activity. A decrease in 1-FFT-activity was observed for late-harvested tubers. Jerusalem artichoke tubers harvested after 50 days of flowering had the highest inulin content and high molecular weight inulin polymers and Jerusalem artichoke tubers harvested after 60 days of flowering had the highest inulin content per rai.

The inulin content of Jerusalem artichokes was strongly influenced by storage temperature. During the storage of Jerusalem artichoke tubers, inulin depolymerization took place causing a decrease in inulin content associated with an increase in fructose and sugar. This effect was associated with an increase in inulin hydrolase (lnH) activity and a decrease in 1-FFT activity. Storage at low temperature (-18 ^{o}C) resulted in a comparably high amount of inulin, and low fructose and sucrose, high activity of 1-FFT and low activity of lnH in Jerusalem artichoke tubers. Specifically, storage

temperatures at -18 ^{o}C retains slow changes in inulin content, fructose, sucrose, kestose, nystose, and activity of 1-FFT degradation more than storage at 0, 5, 10, 15, and 25 ^{o}C.

CO_2 concentration at 0, 5, 10, 15, and 20 percentages affected inulin content, fructose content, sucrose content, kestose, nystose, activity of fructan:fructan 1-fructosyl transferase (1-FFT), and inulin hydrolase (lnH) in Jerusalem artichoke tubers during storage. During storage (30 days), inulin depolymerized resulting in a decrease in inulin content and an increase in fructose, sucrose, kestose, and nystose. This effect associated with an increase in lnH activity and a decrease in 1-FFT activity (P<0.05). Storage at 20% CO_2 concentration best delayed activity of lnH and reduction of inulin content comparing with storage at concentrations of 0, 5, 10, and 15%.

School of Food Technology Student's Signature_____

Academic Year 2013 Advisor's Signature_____

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LIST OF ABBREVIATIONS

ß	=	Beta
^{o}C	=	Degree Celsius
CO_2	=	Carbondioxide
DEAE	=	Diethyaminoethanol
DP	=	Degree of polymerization
%	=	Percentage
1-SST	=	Sucrose:sucrose 1-fructosyl transferase
1-FFT	=	Fructan:fructan 1-fructosyl transferase
1-FEH	=	Fructanexohydrolase
1-KH	=	1-kestose hydrolase
FOS	=	Fructooligosaccharides
RH	=	Relative humidity
O_2	=	Oxygen
G	=	Glucose
GF	=	Sucrose
T	=	Ton
Ha	=	Hectare
SH	=	Sucrose hydrolase
MAP	=	Modified-atmosphere packaging

LIST OF ABBREVIATIONS (Continued)

kPa	=	Kilopascal
kg	=	Kilogram
xg	=	gravity
g	=	Gram
H	=	Hour
lnH	=	Inulin hydrolase
mg	=	Milligram
mm	=	Millimeter
µl	=	Micro liter
min	=	Minutes
ml	=	Milliliter
N_2	=	Nitrogen

CHAPTER I

INTRODUCTION

1.1 Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) originates from North America and is one of the primary sources for inulin in plants. Inulin is a polydispersefructan which has a degree of polymerization (DP) of 2-60 or higher. The fructosyl units are linked by ß (2→1) linkages with an end glucose residue (Hoebregs, 1997; Coussement, 1999). Jerusalem artichoke is a good source of inulin and fructose for both the food industry and pharmaceuticals. Inulin can be used as a raw material for health products, sweeteners, bio-ethanol and animal feed.

In Thailand this plant is not a popular commercial vegetable, but its popularity is increasing in other parts of the world. Studies on the nutritive value of Jerusalem artichoke tubers have shown that they contain some health-promoting components. Tubers contain 20.4-31.9% of dry matter, of which carbohydrates are the main component. Most carbohydrates consist of water-soluble inulin. It has been suggested that inulin is located in the vacuoles on the grounds of its high concentrations (up to 80% of dry weight in Jerusalem artichoke tubers) (Pollock, 1986; Edelman and Jefford, 1968). Soluble carbohydrates, besides inulin, are reducing sugars (fructose and glucose) and sucrose (Cieślik et al., 2005).

Normally, inulin is produced in the roots, tubers, and fruit of plants of the *Asteraceae* family (Campbell et al., 1997). Inulin improves intestinal microflora by

stimulating the growth of bifidobacteria in the human colon and it has been shown to exhibit beneficial health effects by suppressing putrefactive pathogens and reducing the levels of blood glucose, serum cholesterol, phospholipids, and triglycerides (Campbell et al., 1997; Jaime et al., 2000). In addition, it is known to be nontoxic, non-digestible, and sweet. Its energy value is theoretically lower than that of sucrose, since the energy value depends on the extent of absorption in the small intestine and fermentation in the colon. The small human intestine has no enzyme to hydrolyze its glycosidic linkages. Therefore, inulin is considered to be indigestible in the human small intestine (Campbell et al., 1997). Due to its physiochemical properties, sweetening power, and low caloric value, inulin has been recognized as a potential and novel source of functional food.

It is believed that inulin is synthesized in essentially two steps according to the model of Edelman and Jefford (1968), which is based primarily on studies of the tubers of Jerusalem artichoke. The initial step of inulin synthesis is the conversion of two molecules of sucrose into one molecule of 1-kestose and glucose by sucrose:sucrose 1-fructosyl transferase (1-SST). In the next step, 1-kestose is converted to higher DP inulin by fructan:fructan 1-fructosyl transferase (1-FFT). Thus 1-kestose is considered to be an important intermediate for the production of inulin (Fukai et al., 1997). On the other hand, Inulin depolymerization occurs by the action of 1- fructanexohydrolase (1-FEH), which catalyzes the release of free fructose, which in turn is used for the re-synthesis of sucrose for export (Itaya et al., 2002). Therefore, 1-SST, 1-FFT, and 1-FEH enzymes are the key enzymes in the inulin metabolism.

Jerusalem artichoke tubers are difficult to store out of the soil because of the rapid onset of rotting (Frese, 1993). Storage of Jerusalem artichoke tubers at low

temperature (4 ^{o}C) for 34 days also increases the fructo-oligosaccharide content (Kang et al., 1993).

Saengthongpinit and Sajjaanantakul (2005) studied Jerusalem artichoke tubers which were harvested 16, 18, and 20 weeks after planting. They found that tuber maturity contributed to changes in inulin characteristics. A decrease in the polymerized fraction, Degree of Polymerization (DP) >10, with and an increase in fructose and sucrose composition were observed in late-harvesting (20 weeks). The inulin DP distribution profile from tubers, stored at 2 and 5 ^{o}C, significantly changed with increased storage time and temperature. Sucrose and inulin DP 3-10 fractions increased while DP>10 decreased, particularly after 4-6 weeks of storage. Tubers in frozen storage at -18 ^{o}C maintained their DP distribution profiles.

Freezing at -10 ^{o}C, in the field or storage, causes rapid deterioration, but nonlethal freezing at -5 ^{o}C causes little damage. For most fleshy plant products, freezing temperatures can cause damage and the extent of the damage varies with the cultivar, the season, preconditioning, the rate of freezing, and other factors. Tubers can be stored for 6 to 12 months at 0 to 2 ^{o}C and 90 to 95% RH. Some cultivars are much more susceptible to storage losses than others (Kays and Nottingham, 2008). Tubers shrivel readily at low RH and are more likely to decay. Storage of tubers in 22.5% CO_2 + 20% O_2, significantly retarded the rate of inulin degradation, apparently through an effect on enzyme activity (Denny et al., 1944). However, storage of harvested tubers usually results in high losses in quality, caused mainly by rotting, sprouting, freezing, and inulin degradation. 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).

Knowledge of inulin content, inulin profile, and activity of 1-FFT in Jerusalem artichoke tubers during harvesting and postharvest periods is still limited. Therefore, it is important to understand which harvesting and postharvest periods are most suitable for yielding the highest inulin content in Jerusalem artichoke tubers. This could increase the value of the produce.

The objectives of this work are to study the influence of harvesting periods on inulin profiles, inulin content, soluble sugar content and activity of 1-FFT and to study the influence of low temperature and high CO_2 modified atmosphere packaging storage on inulin profiles inulin content, soluble sugar content, the activity of 1-FFT, and the activity of inulin hydrolase (lnH).

1.2 Research objectives

1. To study the influence of harvest periods on inulin and the activity of 1-FFT in Jerusalem artichoke tubers.
2. To study the effects of low temperature on inulin and activities of inulin hydrolase (lnH) and 1-FFT in Jerusalem artichoke tubers during storage.
3. To study the effects of high CO_2 modified atmosphere packaging storage on inulin and activities of lnH and 1-FFT in Jerusalem artichoke tubers during storage.

1.3 Research hypotheses

1. Harvest periods will influence the inulin and soluble sugar contents in Jerusalem artichoke tubers.
2. Activities of 1-FFTwill increase at the peak harvest time and activity of lnH will increase after harvest and during storage.

3. Low temperature and high CO_2 modified atmosphere packaging storage will affect 1-FFT and lnH causing changes in inulin degradation.

1.4 Expected results

The outcome of this research should contribute to a better understanding of knowledge about inulin, activity of 1-FFT in Jerusalem artichoke tubers during harvesting periods, effects of low temperature and high CO_2 modified atmosphere packaging storage of the tubers. It is important to understand which harvesting periods are most suitable, and how low temperatures and high CO_2 modified atmosphere packaging storage yield the highest inulin content in Jerusalem artichoke tubers. Such knowledge will increase the value of the produce.

CHAPTER II

LITERATURE REVIEWS

2.1 Jerusalem artichoke (*Helianthus tuberosus* Linn.) tuber

Jerusalem artichoke (*Helianthus tuberosus*) originates from North America and is one of the primary sources for inulin in plants. Inulin is a polydisperse fructan which has a degree of polymerization (DP) of 2-60 or higher. The fructosyl units are linked by ß (2→1) linkages with an end glucose residue (Hoebregs, 1997; Coussement, 1999). Jerusalem artichoke is a good source of inulin and fructose for both the food industry and pharmaceuticals. Inulin can be used as raw material for health products, sweeteners, bio-ethanol and animal feed. Normally, inulin is produced in the roots, tubers, and fruit of plants of the *Asteraceae* family (Campbell et al., 1997).

Interest in the crop has stemmed from the fact that the storage form of carbon in the Jerusalem artichoke is inulin, a straight chain fructan that is poorly digested by humans. Inulin can be used as a bulking agent in foods when sugar is replaced with an artificial sweetener. The volume previously occupied by sugar is replaced by the low calorie inulin, allowing the total caloric content of the processed product to be greatly reduced. With little reformulation, inulin, though not sweet, functions similarly to sugar, ie., browning reactions, aroma synthesis, textural properties, in many foods. Likewise, inulin, whether ingested as Jerusalem artichoke tubers or as a bulking agent, is a dietary fiber and confers a number of health advantages, eg., it lowers blood cholesterol level; it promotes Bifidobacteria in the large intestine; it reduces blood

sugar level, low density lipoproteins, and triglycerides; and it is beneficial in helping to prevent certain heart diseases (Varlamova et al., 1996). Tuber size and shape are critical quality attributes and are strongly modulated by cultivar and production conditions. Many clones have an irregular tuber surface topography due to branching, which is an undesirable trait.

The tubers are harvested in the late Fall, generally after the first frost. In production areas where harvest can be accomplished throughout winter, the crop can be field-stored and harvested as needed. Elsewhere, harvest is followed by cold storage. There are no existing standard grades. Generally larger tubers with smooth surfaces are preferred. Polyethylene bags are the typical package, though precise recommendations are not established. The parameters for packaging vary with storage temperature, product volume, and other factors. Generally pre-cooling is not required, though placing the tubers under favorable low temperature conditions as soon as possible after harvest is recommended.

The three primary storage options are refrigerated storage, common storage in root cellars, and *in situ* field storage. In common storage in root cellars, champs or pits, cooling is obtained from the natural low temperatures of the outdoor air and/or soil. In the first two options (refrigerated and common storage), tubers are harvested in the Fall and placed in storage. With field storage, however, tubers are left in the ground and harvested as needed. Cold storage is highly effective, but costly. Nevertheless, refrigerated storage is routinely used for seed and fresh market tubers, especially in situations where field storage is not a viable alternative. Root cellars, champs, and pits are used when the tubers must be harvested in the Fall, prior to the ground freezing or other adverse conditions occurring, and refrigeration is not available or prohibitively expense (Kays and Nottingham, 2008).

The selection of *in situ* field storage is dependent upon several factors. Location is the primary determinant in the potential success of *in situ* field storage. Field storage is a viable option in northern hemisphere production areas where cold soil temperatures prevail throughout the winter, but freezing of the soil surface is uncommon. Sandy and well-drained soils are preferred because they allow harvesting throughout the winter. Locations that do not meet these criteria generally require the use of refrigeration or some form of common storage.

The main drawbacks of this crop are related to the harvest time of the mature tubers (generally in December) when the heavy rains make it difficult to use harvest machines in waterlogged fields. Since Jerusalem artichokes also store temporary amounts of fructans in the stalk, some authors (Maijer and Mathijssen, 1991) have suggested using it as a stalk crop, that can be harvested (using a conventional forage harvester) at the beginning of flowering, i.e. August-September in Europe.

Pimsaen et al. (2010) evaluated the effects of cultivar, environment and cultivar x environment interaction on fresh tuber yield, tuber number, and tuber size of Jerusalem artichoke. Fifteen cultivars were evaluated in a randomized complete block design with four replications in nine environments in the Northeast of Thailand. Differences among cultivars were observed for fresh tuber yield, tuber number and weight of individual tuber (tuber size).

Environmental effects contributed to a larger portion of variations in fresh tuber yield, tuber number and tuber size. Although genotype x environmental interactions were also significant, their values were much smaller than genotype main effects for all characteristics. Stability parameters indicated that the growing of cultivars with adaptation to a wide range of environments and cultivars with specific adaptation to specific environments would be possible. Low correlation but significant results were

found for fresh tuber yield and tuber number, indicating that tuber yield in some cultivars was dependent on high tuber numbers. Moreover, JA 89, CN 52867, and JA 37 were the best yielding clones across all environments with yields of 18.9, 18.8 and 18.0 t ha^{-1}, respectively. The cultivars with the best performance for tuber number were JA 37, CN 52867, and JA 89 with tuber numbers of 36, 35, and 34, respectively. It is also noted here that the highest ranks for fresh tuber weight and tuber numbers were very similar (JA 89, CN 52867, and JA 37, respectively). These cultivars showed the highest tuber numbers in at least four environments and specific adaptation to specific environments (Pimsaen et al., 2010).

Pinpong (1997) found that the optimum harvesting stage of Jerusalem artichokes in Thailand was between 18-20 weeks after planting. The 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. Some of these include 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).

2.2 Inulin and soluble sugars

Helianthus tuberosus and *Cichorium intybus* accumulate fructans during their growth. In these fructose polymers a β-2,1glycosidic linkage is formed between adjacent fructose units as shown in Figure 2.1. Fructans are characterized by the degree of polymerization (DP), defined as the number of fructosyl units linked to a terminal glucose. Depending on this, fructans are usually classified as inulins (DP>10) and

fructooligosaccharides (DP<10). Fructan size distribution is a variable that depends on the species and the climatic characteristics among other factors (Pollock and Cairns, 1991). Since fructans are not digestible, they pass through the small intestine into the large intestine without being absorbed. Due to their selective use by *Bifidobacteria* in the large intestine, the energy value of fructans increases because the volatile fatty acids produced in the fermentation are available to the host. Fructans are useful for relief of constipation, improvement of the blood lipid composition, and suppression of intestinal putrefactive substances because of the pH lowering. Fructans of low DP have lower sweetness intensity than sucrose; thus, they can be used in food for diabetics. In addition, the use of fructans in place of sucrose does not replace the beneficial bulking properties provided by sucrose. Because of these factors, fructans have a potential use in the food industry.

The occurrence of β-2, 1-linked fructans in *H. tuberosus* roots has been explained through a mechanism in which 2 enzymes are responsible for their synthesis (Edelman and Jefford, 1968). The initial step of the synthesis is catalyzed by the sucrose:sucrose 1-fructosyl transferase (1-SST, EC 2.4.1.99) reaction in which an intermediate trisaccharide, 1-kestose, is formed from two sucrose (GF) molecules as shown in Figure 2.2 (GF + GF $\longrightarrow$ GF2 + G); in this reaction, one of the sucrose molecules is the acceptor of the fructosyl unit, while the other is the donor. Fructans of higher DP are formed when the fructosyl transfer takes place between fructan molecules (GFn + GFm $\longrightarrow$ GFn-1 + GFm+1) in the reaction catalyzed by the fructan: fructan 1-fructosyl transferase (1-FFT, EC 2.4.1.100). While high-molecular-weight fructans are effective acceptors and donors of fructosyl units, sucrose can only act as an acceptor ($m \geq 1$, $n > 1$).

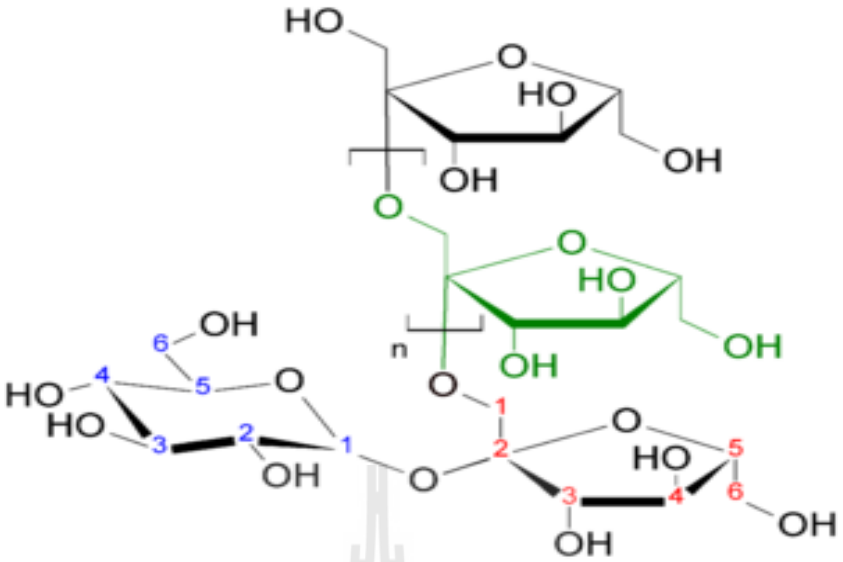

Figure 2.1 The chemical structure of inulin.

Source: Kay and Nottingham (2008).

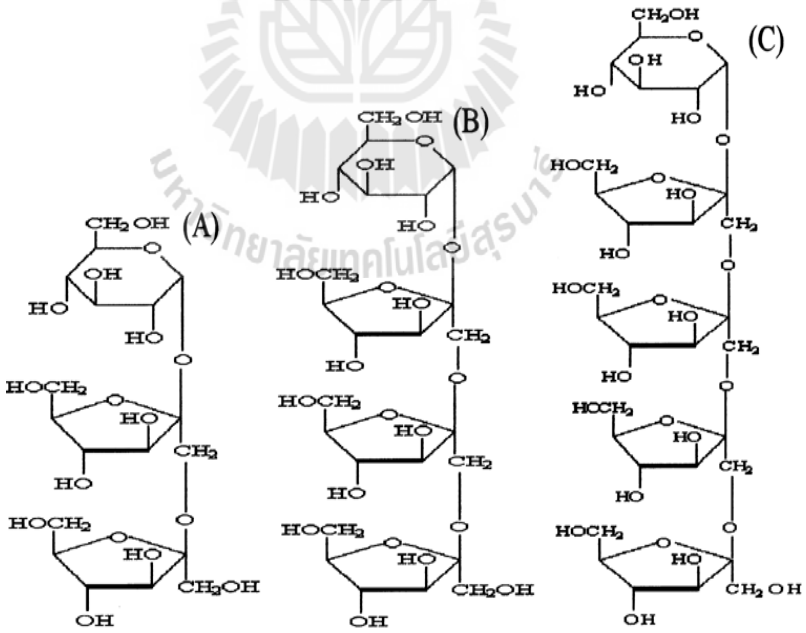

Figure 2.2 The chemical structure of frutooligosacarideos : 1-ketose (A), nystose (B), and frutofuranosilnystose (C).

Source: Kay and Nottingham (2008).

This model has been questioned since the *in vitro* synthesis of high-molecular-weight fructans has not been demonstrated. Cairns (1993) has categorized the catalytic action of 1-FFT as "non-synthetic fructosyl transfer" since this does not catalyze the *de novo* net synthesis of fructans. It has been claimed that the function of the 1-FFT would be to control the fructan size distribution (Cairns, 1993). On the other hand, even though 1-SST activity has been reported in many plant extracts, the major product in all cases being 1-kestose, the sucrose concentration used in those experiments may have led to problems. This is because the acid invertases (β-fructofuranosidase, EC.3.2.1.26), which are widespread in plant tissue, catalyze not only sucrose hydrolysis, but also the fructosyl transfer (Van den Ende and Van Laere, 1993) in concentrated sucrose solutions. In this case, the acid invertases act on either of the two sucrose molecules, forming the trisaccharide (GF+GF → GF2+G), or on sucrose and fructan molecules (GF + GFn → GFn+1 + G), increasing the fructan DP. It has been suggested that the role of fructan metabolism is to control the sucrose content inside the vacuole (Housley and Pollock, 1993). From the enzymatic reactions shown above, both enzymes, 1-SST and 1-FFT, reduce the sucrose content. Glucose formed in the reaction catalyzed by the 1-SST would be phosphorylated and resynthesized into sucrose, a process that allows the synthesis of more fructan molecules. The hydrolysis of fructans in plants is catalyzed by fructan hydrolases. However, this hydrolysis has only been reported through enzymes having fructan 1-exohydrolase (1-FEH, EC 3.2.1.80) activity. Partial purification of enzymes extracted from roots has identified 2 forms of 1-FEH in *H. tuberosus* (Edelman and Jefford, 1968) and *C. intybus* (Claessens et al., 1990). Both isolated forms are inactive against sucrose but are inhibited by it (Edelman and Jefford, 1968; De Roover et al., 1999). It has been shown that extractable activities of fructan exohydrolases rise during periods of fructan

mobilization; in *H. tuberosus*, this activity shows seasonal variation (Edelman and Jefford, 1964).

There is a great potential for the use of fructans as a raw material in a number of interesting food and non-food applications (Hidaka et al., 1991). Jerusalem artichoke is a native of North America and is a close relative of the common sunflower. A tuber yield of 46-60 t ha^{-1} has been reported (Kiehn and Chubey, 1993). This crop has a good potential for production of fructose and as a feed stock for production of ethanol. The top growth provides an excellent animal feed. Jerusalem artichoke is known as a crop with high production potential. Under the climatic conditions of Northern Europe, the potential yield from early cultivars is about 10 tons DM ha^{-1} (Zubr and Pedersen, 1993). The yield abilities of Jerusalem artichoke and chicory are equal or even surpass the productivity of sugar beet. In chicory, inulin yield varies from 8 to 12.2 t ha^{-1} comprising about 57 % of total dry matter and 74% of root on an average. The inulin yield of Jerusalem artichoke varies from 4.0 to 6.7 t ha^{-1}. This isan average of about 28% of total dry matter and 71% of tuber weight (Meijer et al., 1993).

2.3 Fructan metabolism

Edelman and Jefford (1968) described the enzyme systems of 1-SST and 1-FFT for the synthesis of fructan. On storing chicory roots for forcing the sprouting of Jerusalem artichoke tubers or during flowering in chicory, l-FEH becomes active. An important feature of fructan storing tissues is that glucose, the product of 1-SST, does not accumulate. Obviously glucose to sucrose conversion either by sucrose synthase (SS) or sucrose phosphate synthase (SPS) could be important. Possibly some of the sucrose is also hydrolyzed by invertase in these tissues.

2.3.1 Sucrose:sucrose 1-fructosyl transferase (1-SST)

Sucrose:sucrose 1-fructosyl transferase (1-SST) is a key enzyme in the biosynthesis of inulin and it catalyzes the synthesis of 1-kestose (GF2) from sucrose by trans fructosylation reaction. Activity of SST in roots of young plants of chicory synthesizing large amounts of fructans was higher than in mature roots suggesting a role for 1-SST in determining sink strength (Ameziane et al., 1995). An enzyme purified from chicory root could accomplish the synthesis of GF2, while at low concentration it hydrolyzed sucrose; therefore this enzyme was designated as invertase (Van den Ende and Van Laere, 1993). 1-SST has been purified to homogeneity from tubers of *H. tuberosus* (Praznik et al., 1990).

2.3.2 Fructan:fructan 1-fructosyl transferase (1-FFT)

Fructan:fructan 1-fructosyl transferase (1-FFT) from *H. tuberosus* has also been investigated by Luschner et al. (1993a). They focused on the transfer of fructosyl units from various oligofructans to GF by 1-FFT isolated from dormant tubers of *H. tuberosus*. These characteristics probably indicated the role of 1-FFT in mobilization of fructans during dormancy and sprouting. The rate of self transfer with GF2, GF3, and GF4 increased linearly with oligosaccharide concentration and did not fully saturate even at very high substrate concentrations. 1-FFT can mediate the synthesis of a range of oligofructans from GF2. However, the kinetics of a self transfer reaction cannot be resolved as the substrate concentration as the donor and acceptor sites cannot be varied independently (Koops and Jonker, 1994). 1-FFT enzymes purified from dandelion (Luscher et al., 1993b), Jerusadem artichoke (Koops andJonker, 1994; Luscher et al., 1993a) and chicory (Van den Ende et al., 1996b) can transfer fructose from higher fructans to sucrose or lower fructans. 1-FFT purified from chicory roots showed β-fructosidase activity especially at higher temperature and low

sucrose concentrations (Van den Ende et al., 1996a). An effective way to decrease its sucrose hydrolytic activity was to incubate the assay mixture at 0 ^{o}C. However, even at this temperature some β-fructosidase activity was observed. Glucose was not a good acceptor of fructosyl moiety.

1-SST and 1-FFT, the two enzymes needed in fructans synthesis, differ in their chromatographic and electrophoretic behaviors and also enzymic properties. For example, 1-FFT is not able to catalyze the initial step of fructan synthesis.Whereas1-SST is not able to catalyze the synthesis of fructan polymers with a DP higher than 5. However, 1-SST is able to transfer fructosyl units as effectively as 1-FFT between GF2, GF3 or GF4 molecules. Although 1-SST and 1-FFT have some overlapping activity (both enzymes can catalyze the formation of GF3 and GF4); GF3 and GF4 synthesis is more efficiently catalyzed by 1-FFT (Koops and Jonker, 1994).

Moreover, it was found that fructan patterns synthesized *in vitro* strongly depend on the enzyme concentration used (Cairns, 1995). Using purified chicory root 1-SST and 1-FFT, a higher enzyme concentration resulted in a higher relative abundance of a larger degree of polymerization fructans in the reaction mixtures. The final fructan pattern obtained *in vitro* is a function of the (1-SST + 1-FFT)/sucrose ratio, suggesting that the latter ratio *in situ* could affect the highly variable tissue- or species-specific pattern of fructans produced *in vivo* (Van den Ende and Van Laere, 1996).

2.3.3 Fructan 1-exohydrolase (1-FEH)

Edelman and Jefford (1964) first reported the separation of two fructan hydrolytic activities from Jerusalem artichoke tubers by DEAE cellulose chromatography. There were 1-FEH I and 1-FEH II. However, no significant difference in the properties of these two hydrolase was observed. These enzymes were

inactive on sucrose. Enzymes preparation were more active on DP5>DP4>DP3. The optimum chain length for maximal activity was DP 5-8. Sucrose was the non-competitive inhibitor. However, sucrose had no inhibitory effect on fructan hydrolase of barley leaves or roots of *Taraxacumo fficinale*. When the tubers of Jerusalem artichoke are dormant, 1-SST activity disappears and 1-FEH appears. This enzyme removes fructosyl residues from the end of fructan chains, thus initiating depolymerization and remobilization of stored fructans (Claessens et al., 1990).

Fructan degradation in chicory starts at the end of the growing season when 1- SST has almost disappeared, 1-FFT activity is still high and 1-FEH is induced. In the absence of 1-SST,1-FFT will use any incoming sucrose as an acceptor substrate for fructosyl units from larger fructans resulting in a reduced average degree of polymerization and an increase in molar concentration of fructans (although not of fructan weight). Fructans transfer to free fructose has a similar effect. The increase in molar concentration might contribute to cold resistance and/or more efficient inulin breakdown (by increased substrate concentration). Perhaps the lower degree of polymerization of the resulting fructans contributes to a greater affinity of 1-FEH towards its substrates (Van Laere and Van den Ende, 2002).

2.4 Changes in inulin and soluble sugars during storage

Jerusalem artichoke tubers are difficult to store above the soil because of the rapid onset of rotting. Therefore, the crop must be harvested according to the daily capacity of the processing facilities (Frese, 1993). Early-harvested tubers contain a greater amount of highly polymerized sugar fractions, which offer more industrial value than late-harvested tubers or those which have been in storage (Schorr-Galindo

and Guiraud, 1997). Degradation of inulin to sucrose and fructo-oligosaccharides is highest after cold shock. Storage of Jerusalem artichoke tubers at a low temperature 4 ^{o}C for 34 days also increases the fructo-oligosaccharide content (Kang et al., 1993). During the cold storage of harvested roots not only did the fructose increase significantly, but the second fructan series also increased rapidly during the first 5 weeks. The rapid increase of fructans in the second fructan series coincided with a reduction in mean DP and a decrease in total fructan content (Ernst et al., 1996). Tubers can be stored for 6 to 12 months at 0 to 2 ^{o}C and at 90 to 95% RH. Some cultivars are much more susceptible to storage losses than others. Tubers shrivel readily at low RH and are more likely to decay. Water loss accounts for the majority of postharvest losses during retail sales. Products should be displayed in refrigerated display cases when not packaged under high RH conditions such as that afforded by mist systems. Jerusalem artichoke tubers can withstand low temperatures without damage. Tubers freeze at temperature below -2.2 ^{o}C, and temperatures around -10 ^{o}C, whether in the field or storage, causes rapid deterioration. Significant chemical and physical alterations occur in the plasma membrane. Nonlethal freezing temperature ($\geq$ -5 ^{o}C), however, result in little damage. As with most fleshy plant products, the temperature at which freezing damage occurs and the extent of the damage varies with cultivars, seasons, preconditioning, rates of freezing, and other factors (Kays and Paull, 2004). Tubers are not sensitive to ethylene. Storage losses are due primarily to desiccation, rotting, sprouting, freezing, and inulin degradation. Desiccation remains a significant storage problem even though losses can be fairly easily circumvented with proper storage conditions. Storage at high RH is essential because tubers lack a corky surface layer similar to that found on potatoes to reduce water loss, and they have a thin, easily damaged surface that permits rapid water loss. While it is beneficial for

some produce (Kays, 1997), γ-irradiation greatly accelerates inulin degradation and is of little storage value.

Storage rotting is a serious problem (Barloy, 1988) and higher storage temperatures result in greater loss. Approximately 20 organisms causing storage rotting have been isolated from Jerusalem artichoke tubers. The organisms most frequently isolated were *Botrytis cinerea* Pers. and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill., though *R. stolonifer* and *Sclerotinia sclerotiorum* (Lib.) de Bary are the most serious organisms which cause rotting at low storage temperatures. During storage, tubers undergo significant alterations in carbohydrate chemistry which, depending upon the intended use, can have a pronounced effect on quality. Inulin is not one compound, but a series of molecules of varying chain length that begin to depolymerize during storage (Schorr-Galindo and Guiraud, 1997), whether harvested or left *in situ*. The degree of polymerization is critical for uses such as fat replacement or high fructose syrups. With the former, as the chain length decreases, the ability of inulin to mimic a lipid diminishes. Likewise, with progressive depolymerization, the ratio of fructose:glucose decreases and upon hydrolysis yields a progressively less pure fructose syrup. For example, during winter storage the fructose:glucose ratio decreases from 11 to 3 (Schorr-Galindo and Guiraud, 1997). Thus, syrups derived from stored tubers contain a lot more glucose.

In work conducted by Saengthongpinit and Sajjaanantakul (2005), 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 polymerized fractions (degree of polymerization, 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 ^{o}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. Tubers in frozen storage at -18 ^{o}C maintained their DP distribution profiles.

Ishiguro et al. (2010) found that burdock roots stored for six weeks at three temperatures, 0, 15, and 20 ^{o}C, and the activity of sucrose hydrolase (SH), 1-kestose hydrolase (1-KH), inulin hydrolase (lnH), 1-SST, and 1-FFT were assessed. Simultaneously, total fructooligosaccharides (FOS), total inulooligosaccharides (IOS) and inulin were also assessed during storage. During storage, SH showed an irregular variation at 15 and 20 ^{o}C, and was significantly higher at 0 ^{o}C showing a continuous increase during the storage period. 1-KH decreased progressively at 15 and 20 ^{o}C, but showed a sharp increase at 0 ^{o}C after two weeks and decreased afterwards. lnH showed a different although more regular pattern by decreasing progressively at 0, 15, and 20 ^{o}C. However, the decrease was more significant at 15 and 20 ^{o}C during the first two weeks, while at 0 ^{o}C the decrease was significant after four weeks storage. 1-SST and 1-FFT activities decreased progressively in a pseudo-linear regression during storage, and showed similar patterns. At 15 and 20 ^{o}C, the total FOS increased during the first three weeks then decreased, while at 0 ^{o}C FOS increased progressively during storage. Total IOS increased during storage, however, and this increase was much higher at 0 ^{o}C than that observed at 15 and 20 ^{o}C. Inulin content decreased during storage and final content was lower at 20 ^{o}C. The inulin to total FOS plus total IOS ratio decreased sharply during the first two weeks of storage, then progressively during the last four weeks, while the ratio of hydrolyzing to synthesizing activities was quite stable at 15 and 20 ^{o}C, but showed a peak at 0 ^{o}C after two weeks. These results suggest that

carbohydratemetabolism in stored burdock depends partly on temperature and partly on other physiological factors.

Cabezas et al. (2002) observed the inulin, sucrose, fructose, and glucose contents in tubers of *H. tuberosus* (Jerusalem artichoke) and *C. intybus* stored at different temperatures (-18, 4, and 18 ^{o}C) after harvesting. Inulin content in both tubers decreased during storage. In *C. intybus* this decrease associated with increases in glucose and fructose contents. In *H. tuberosus* the fructan fraction having molecular weight between 800 and 1,200 increased after sucrose reached its maximum content in tubers stored at 4 ^{o}C. The fructose-to-glucose ratio was observed in *H. tuberosus* tubers harvested at different times; samples from plants subjected to different fertilization treatments were used. This parameter can be used for choosing the harvest date since it is related to the disappearance of the fructan fraction having molecular weight higher than 1200.

Bohm et al. (2005) found that dry heating of inulin from chicory for up to 60 min at temperatures between 135 and 195 ^{o}C resulted in a significant degradation of the fructan ranging from 20 to 100%. The choice of the analytical method had a significant influence on inulin quantification, especially in heat-treated samples. The amount of inulin found after thermal treatment measured as fructose after acidic hydrolysis was significantly higher compared to corresponding data obtained by a method based on enzymatic hydrolysis. Using high-performance anion-exchange chromatography with pulsed amperometric detection as well as high-performance thin-layer chromatography, it was found that thermal treatment of inulin leads to a degradation of the long fructose chains and formation of new products.

2.5 Modified-atmosphere packaging (MAP)

Modified atmosphere packaged (MAP) products can be found in almost every aisle of the supermarket today. Billions of packages of refrigerated, frozen and shelf stable foods are sold worldwide every year that are packaged with this proven technology. This array of food products includes potato chips and other snack foods; case-ready fresh meats; fresh produce; hot dogs and other processed meat products; pizza dough and other bread and bakery products; and items as diverse as coffee, fresh pasta and cheese. MAP is a preservation technique that prolongs product shelf life, which directly and positively impacts the profitability and marketability of a food business. MAP of fresh fruits and vegetables refers to the technique of sealing actively respiring produce in polymeric film packages to modify the O_2 and CO_2 levels within the package atmosphere. It is often desirable to generate an atmosphere low in O_2 and/or high in CO_2 to influence the metabolism of the produce being packaged, or the activity of decay-causing organisms to increase storability and/or shelf life. For some produces, modifying both O_2 and CO_2 may be desirable, and indeed, altering the O_2 level automatically alters the CO_2 level. In addition to atmosphere modification, MAP vastly improves moisture retention, which can have a greater influence on preserving quality than O_2 and CO_2 levels.

2.6 Gases used in modified atmosphere packaging

The three main gases used in modified atmosphere packaging are CO_2, O_2, and N_2. The choice of gas is very dependent upon the food product being packed. Used singly or in combination, these gases are commonly used to balance safe shelf-life extension with optimal organoleptic properties of the food.

2.6.1 Carbon dioxide (CO_2)

Carbon dioxide is a colorless gas with a slight pungent odour at very high concentrations. It is an asphyxiant and it is slightly corrosive in the presence of moisture. CO_2 dissolves readily in water (1.57 g/kg at 100 kPa, 20 ^oC) to produce carbonic acid (H_2CO_3) that increases the acidity of the solution and reduces the pH. This has significant implications for MAP of foods. The high solubility of CO_2can result in the collapse of packaging due to the reduction of head space volume. In some MAP applications, the collapse of packaging is favoured, for example, in flow wrapped cheese for retail sale.

CO_2 is an important chemical compound in MAP of fruits and vegetables since it lowers the rates of respiration, ethylene production and ripening and inhibits microbial growth. To gain an insight into the mechanisms of the above-mentioned inhibitions, the chemistry of CO_2 in water should be known (Sandhya, 2010).

2.6.2 Oxygen (O_2)

Oxygen is a colorless, odorless gas that is highly reactive and supports combustion. It has a low solubility in water (0.040 g/kg at100 kPa, 20 ^oC). Oxygen promotes several types of deteriorative reactions in foods including fat oxidation, browning reactions and pigment oxidation. Most of the common spoilage bacteria and fungi require oxygen for growth. Therefore, to increase the shelf life of foods the atmosphere in the packaging should contain a low concentration of residual oxygen (Sandhya, 2010).

2.6.3 Nitrogen (N_2)

Nitrogen is a relatively un-reactive gas with no odor, taste, or color. It has a lower density than air, it is non-flammable and has a low solubility in water (0.018 g/kg at 100 kPa, 20 ^oC) and other food constituents. Nitrogen does not support the

growth of aerobic microbes and therefore inhibits the growth aerobic spoilage but does not prevent the growth of anaerobic bacteria. The low solubility of nitrogen in foods can be used to prevent the collapse of packaging by including sufficient N_2 in the gas mix to balance the volume decrease due to CO_2 going into solution (Sandhya, 2010).

2.7 Modified-atmosphere packaging of fresh fruits and vegetables

Modified-atmosphere packaging (MAP) of fresh fruits and vegetables is a process involving (1) the introduction of whole, separated, segmented or cut products into a package system with either air or an input gas mixture having a specific MA composition, as the initial surrounding microatmosphere; (2) the closure (sealing) of the package system; and (3) the refrigerated storage of the packaged product at -1 to 12 ^{o}C. During the first few days of storage, products with an oxygen-containing microatmosphere progressively convert the O_2 to CO_2 by means of respiration reactions (Kader, 1985; Prince, 1989). The objective in the design of the MA package system is to achieve and maintain a suitable equilibrium MA (low O_2 and elevated CO_2 contents) around the produce during storage for optimum retention of quality attributes and inhibition of microbial growth. Reduced O_2 and elevated CO_2 in the microatmosphere around fruits or vegetables reduce respiration rate, ripening and microbial growth; the effects of low O_2 and high CO_2 contents are additive for both respiration and ripening control (Kader, 1985; Zagory and Kader, 1988). Unsuitable MA may be responsible for inducing physiological damage, preventing wound healing, enhancing the senescence process and producing off-flavour compounds. Generally O_2levels lower than about 1% in the microatmosphere will bring about anaerobic respiration (Boersig et al., 1988). CO_2 levels of 10% and above can inhibit the growth

of spoilogens, but may cause physiological damage to CO_2 sensitive commodities (Goorani and Sommer, 1981; Kader, 1985).

When packaging vegetables and fruit, the gas atmosphere in the package is not air (O_2-21%; CO_2-0.01%; N_2-78%) but consists usually of a lowered level of O_2 and a heightened level of CO_2. This kind of package slows down the normal respiration of the produce and so prolongs its shelf life. There are many factors which effect modified atmosphere packaging of fresh produce. Movement of O_2, CO_2, and C_2H_4 in produce tissues is carried out by the diffusion of gas molecules under a concentration gradient. Different commodities have different amounts of internal air space (potatoes 1-2%, tomatoes 15-20%, apples 25-30%). A limited amount of air space leads to an increase in resistance to gas diffusion. The evaluation of these gases (O_2, CO_2, and C_2H_4) in three varieties of apricots stored at 10 ^oC under four plastic films of different permeabilities has been studied (Pretel et al., 2000).

One of the primary effects of MAP is a lower rate of respiration, which reduces the rate of substrate depletion. Ethylene (C_2H_4) is a natural plant hormone and plays a central role in the initiation of ripening, and is physiologically active in trace amounts (0.1 ppm). C_2H_4 production is reduced by about half at O_2 levels of around 2.5%. This low O_2 retards produce ripening by inhibiting both the production and action of C_2H_4. Modified atmosphere packaging of cabbage stems also showed improvement in quality (Escalona et al., 2006). Also metabolic processes such as respiration and ripening rates are sensitive to temperature. Biological reactions generally increase two to three-fold for every 10 ^oC rise in temperature. Therefore temperature control is vitally important in order for a MAP system to work effectively. Film permeability also increases as temperature increases, with CO_2 permeability responding more than O_2 permeability. Low RH can increase transpiration damage and lead to desiccation, increased

respiration, and ultimately an unmarketable product. One serious problem associated with high in-package humidity is condensation on the film that is driven by temperature fluctuations. A mathematical model was developed for estimating the changes in the atmosphere and humidity within perforated packages of fresh produce (Lee et al., 2000). The model was based on the mass balances of O_2, CO_2, N_2, and H_2O vapours in the package. Also a procedure to maintain the desired levels of O_2 and CO_2 inside packages that are exposed to different surrounding temperatures was designed and tested (Silva et al., 1999). For most commodities light is not an important influence in their post-harvest handling. However, green vegetables in the presence of sufficient light, consume substantial amounts of CO_2 and produce O_2 through photosynthesis. Shock and vibration leads to damage to produces cells which causes an increase in respiration and may lead to enzymes being released that will cause browning reactions.

For MAP of fruits and vegetables, package systems may consist of either low-, medium- or high-barrier films. Whole fruits and vegetables under MAP require sufficient O_2 (generally above 1 %) and adequate CO_2 (up to the CO_2 tolerance level) in the microatmosphere of a package system to provide near optimum preservation of fresh commodities without anaerobic respiration. For ongoing oxygen flow into the microatmosphere of commodity-containing package system, a low-barrier flexible film with a specific O_2 transmission rate is required. Respiratory CO_2 also must be diffused out of the package system through the plastic wall. The influx of O_2 and the efflux of CO_2 are governed by the permeability of the plastic film, thickness and surface area of the film, and the partial pressure gradients of the gases inside and outside the package system (Kadar et al., 1989; Labuza and Breene, 1989).

When produce is present in a package system within which an optimum equilibrium MA exists, the following benefits would be expected (Agriculture Canada, 1990; Lioutas, 1988)

1) Reduction of respiration rate
2) Lowering of ethylene production
3) Inhibition of the initiation of ripening
4) Decrease in rate of ripening and senescence
5) Reduction of tissue water loss and maintenance of cell turgidity
6) Minimization of nutrient decomposition
7) Inhibition of microbial growth and spoilage
8) Reduction of specific physiological disorders such as chilling injury
9) Maintenance of membrane and cell wall integrity and natural barriers against microbial invasion
10) Encouragement of wound healing

It is important to recognize that while atmosphere modification can improve the storability of some fruits and vegetables, it also has the potential to induce undesirable effects. Fermentation and off-flavors may develop if decreased O_2 levels cannot sustain aerobic respiration (Kays, 1997). Similarly, injury will occur if CO_2 exceeds tolerable levels. Ranges of non-damaging O_2 and CO_2 levels have been published for a numbers of fruits and vegetables (Kader, 1997; Kupferman, 1997; Beaudry, 1999, 2000). Horticultural crops differ in their tolerance of O_2 and CO_2.The range of O_2 and CO_2 levels for fruits and vegetables can overlap, or be distinct. The atmosphere generated by MAP delays ripening of certain subtropical-tropical fruits, including mangos (Kader, 1994).

Jerusalem artichoke tubers can be stored without great losses at 2.5 ^{o}C for five months. Field-dug tubers with rot or damaged tissue were removed; the remainder were surface-sterilized in a 0.025 percent sodium hypochlorite solution. After air drying, the tubers were placed in metal cans, moved to a 2.5 ^{o}C storage chamber, and cooled rapidly. The cans were sealed and partially evacuated to reduce O_2 levels to 2 to 5 percent. The atmosphere in the cans was monitored frequently and maintained at 10 to 15 percent CO_2, and 2 to 5 percent O_2. Approximately 79 percent of the tubers survived five months without disease symptoms: the remainder were partially rotted by an unidentified fungus. Some growers have successfully stored tubers at 0 ^{o}C after packaging the surface-dried tubers in plastic bags and cooling them rapidly (Sachs et al., 1981). Storage of Jerusalem artichoke tubers in 22.5% CO_2 + 20% O_2, significantly retarded the rate of inulin degradation, apparently through an effect on enzyme activity (Denny et al., 1944).

CHAPTER III

MATERIALS AND METHODS

3.1 Influence of harvesting period on inulin in Jerusalem artichoke tubers

3.1.1 Preparation of Jerusalem artichoke tubers

CN 52867 cultivar of Jerusalem artichoke tubers with high inulin and high yield (Pimsaen et al., 2010) was grown at the Khon Kaen University Farm, Khon Kaen, Thailand from July to November, 2010. It was harvested at different maturity at 30, 40, 50, 60, and 70 days after flowering (DAF). All tubers were washed and soaked in 0.038 M sodium hypochlorite for 30 minutes to eliminate soil and reduce micro-organisms. Some samples were selected for fresh tuber analysis on the harvest date, then freeze dried for preservation to prevent them from brown rot. A Jerusalem artichoke powder was obtained by peeling, slicing, freeze dried, and milling and then the whole sample was passed through a 0.15 mm sieve. The prepared sample was stored in a dry container at -20 ^{o}C for further use. These tubers were analyzed for their inulin profiles, inulin and soluble sugar contents mainly reducing sugars (fructose and glucose) and sucrose.

Appropriate dilutions of a standard solution containing glucose, fructose, sucrose, 1-kestose, nystose, and inulin were used for calibration. Chromatographic peaks from high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) were identified by comparing sample retention

times with those of known standard mixtures. The retention times and response factors for sugars were verified daily with a standard solution.

3.1.2 Determination inulin profiles

One-hundred and fifty grams of tubers were taken randomly from each pack and chopped into small pieces with a homogenizer. Inulin was extracted with hot deionized water using a modified method that of VanWaes et al. (1998). Eighty-five grams of deionized water were added to flasks with 11.5 g of crushed tubers, and the slurry was shaken at 130 rpm at 85 ^{o}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 centrifuged for 20 min at 10,000*g*. This extraction was repeated three times. Supernatants were stored at -20 ^{o}C and, prior to inulin profile analysis, were thawed in a water bath at 40 ^{o}C. Inulin profiles were analyzed by high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD), using a DionexBioLC (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 an appropriate concentration with deionized water, and filtered through a 0.20 µm Satorius cellulose acetate membrane before injection. The injection volume used was 25 µl by autosampler (AS50). A CarboPac PA200 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 was150 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 at 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 at: 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 used at 0.1 V. The other potentials were used to clean the electrodes. High and low potential were used to eliminate the gold oxide on the surface of the working electrode to avoid electrode fouling (Cataldi et al., 2000). The relative percentage DP composition of inulin was calculated based on the peak area from the chromatogram, as integrated by the Chromeleon, software version 6.2 from Dionex.

3.1.3 Determination of water soluble sugars, kestose, and nystose

Soluble sugars were extracted with hot deionized water using of the method of Raessler et al. (2008) with modification. Dried and ground tuber samples (500 mg) was weighed in 200 ml flasks. Distilled water (50 ml) was added, and flasks were shaken in water bath at 85 ^{o}C for 30 min. Flasks were cooled to room temperature and water extracts were centrifuged at 10,000*g* for 20 min to obtain clear solution (This extraction was repeated three times). After centrifugation and separation of phases, the liquid phase from each extraction was collectively transferred to another flask and solutions were transferred to a 200 ml volumetric flask and filled to the mark with distilled water. Aliquot of this solution was transferred to sample vials. Fructose, glucose, sucrose, kestose, and nystose were analyzed by HPAEC-PAD, using a DionexBioLC (Sunnyvale, CA, USA) in the same maner as in 3.1.2. The soluble sugars was calculated based on the peak area from the chromatogram, as integrated by the Chromeleon, software version 6.2 from Dionex.

Appropriate dilutions of a standard solution containing glucose, fructose, sucrose, 1-kestose, and nystose were used for calibration. Chromatographic peaks from HPAEC-PAD were identified by comparing sample retention times with those of known standard mixtures. The retention times and response factors for sugars were verified daily with a standard solution.

3.1.4 Determination of inulin content

Inulin was extracted using hot deionized water using a method modified from those of Raessler et al. (2008) and Lingyun et al. (2007). Dried and ground tuber samples (500 mg) were weighed in 200 ml flasks. Distilled water (50 ml) was added. The steps for the preparation of the samples before centrifugation were the same as that described in section 3.1.3. After centrifugation, the liquid phase (10 ml) was transferred to another centrifugation tube. To this solution, 340 µL HCl 30% was added to make an overall acid concentration of 1%. Tubes were again placed in a shaking water bath at 85 ^{o}C for 1 h for complete hydrolysis of inulin. After cooling the centrifuge tubes to room temperature, solutions were transferred to 50 ml volumetric flasks and filled to the mark with distilled water.

Aliquot of this solution was transferred to sample vials. Products from inulin hydrolysis, i.e. fructose and glucose were analyzed by HPAEC-PAD, using a DionexBioLC (Sunnyvale, CA, USA), equipped with an ED 50-pulsed electrochemical detector with gold, working electrode and silver chloride as a reference electrode. The water extracts were diluted to an appropriate concentration with deionized water, and filtered through a 0.20 µm Satorius cellulose acetate membrane before injection. The injection volume used was 25 µl by autosampler (AS50). A CarboPac PA200 column (2 mm×250 mm) with guard column was used with eluents A at a flow rate of 0.25 ml min^{-1}. Eluent A was 150 mM sodium hydroxide, The concentration of sodium

hydroxide was kept constant to ensure an optimal pH for the analysis (Van Waes et al., 1998). The system was equilibrated with eluent A for 10 min before analysis. The elution was at 0-30 min with 100% eluent A. The soluble sugars was calculated based on the peak area from the chromatogram, as integrated by the Chromeleon, software version 6.2 from Dionex.

Appropriate dilutions of a standard solution containing glucose, fructose, were used for calibration. Chromatographic peaks from HPAEC-PAD were identified by comparing sample retention times with those of known standard mixtures. The retention times and response factors for sugars were verified daily with a standard solution.

However, for correct quantification of inulin, the amount of water-extractable fructose was directly subtracted from the fructose concentration measured in the inulin hydrolysis. The amount of fructose from the sucrose hydrolysis was calculated according to the equilibrium:

$$\underset{\text{Mw } 342.3}{\text{sucrose}} + H_2O = \underset{\text{Mw } 180}{\text{fructose}} + \text{glucose}$$

Using eq 1 gives the amount of fructose from the hydrolysis of sucrose:

$$\text{sucrose (mg/L)} / 342.3 \ ) = \text{sucrose (mmol)} * 180$$

$$= \text{fructose (mg/L)} \qquad (1)$$

The fructose content from inulin hydrolysis was then calculated according to eq 2 (mg/L):

fructose (inulin) = fructose (hydrolysis) - [fructose (water extractable) + fructose (sucrose hydrolysis)] (2)

The inulin content of the sample was finally determined by eq 3:

$$\text{Inulin} = 0.91 * \text{fructose (inulin)} \quad I = 0.91 * F \tag{3}$$

The factor 0.91 takes into account the hydrolytic equilibrium between inulin and the monomer; because, it arises from the uptake of one molecule of water per fructose residue in the polysaccharide during hydrolysis. If water extracts reveal the presence of substantial amounts of oligosaccharides, such as raffinose, their fructose content has to be taken into consideration. For this purpose, an Excel program was used to carry out the necessary calculations (Raessler et al., 2008). Inulin data in this study were reported and discussed on the basis of the calculations used in the Excel program.

3.1.5 Enzyme extraction

Enzyme extraction was conducted according to the method of Henson and Livingston (1996); Fukai et al. (1997). All operations were carried out on ice. Tuber tissues (5 g) were homogenized in 30 ml of ice-cold 10 mM sodium phosphate buffer containing 1mMdithiothreitol (pH 6.5) using a homogenizer (ACE homogenizer-AM4 base with motor 250×245×380(II) mm , Tokyo, Japan) at full speed for 230-s intervals. The homogenate was squeezed through 3 layers of cheesecloth and centrifuged at 12,000*g* for 20 min. This extraction was repeated three times. The supernatant from each extraction was then collected in a volumetric flask and then filled up to 100 ml with the extraction buffer. An aliquot of 10 ml was concentrated to 1mL by centrifugation at 3000*g* using an AmiconUltra15 with 10,000 MWCO filter (Amicon Bioseparation, Millipore, Bedford, MA, USA). The concentrate was diluted to 10 ml with the extraction buffer then re-concentrated as described previously. The process was repeated three times. This enzyme extract was used for enzyme assays.

3.1.6 Assay of fructan:fructan 1-fructosyl transferase (1-FFT) activity

Fructan:fructan 1-fructosyl transferase (1-FFT) activity was studied according to the method modified from these of Ishiguro et al. (2010) and Imahori et al. (2010). One unit of 1-FFT activity was defined as the amount of the enzyme which catalyzed the fructosyl transfer from 1-kestose to another 1-kestose to synthesis 1nmol of nystose in 1min under the conditions described below. A mixture solution of enzymes (100 µL), 0.5 % 1-kestose solution (200 µL) and McIlvaine buffer (pH 5.5, 200 µL) was incubated at 30 ^{o}C for 3 h. The reaction was stopped by heating in boiling water for 10 min. The activity of 1-FFT was calculated from the amount of nystose formed and analyzed by HPAEC in the same maner as in 3.1.2.

3.1.7 Total soluble solids and dry matter

The total soluble solids of Jerusalem artichoke tubers were measured by hand refractometer (NOW-507-I, BRIX 0-32%, Tokyo, Japan). Tuber tissues (50 g) were homogenized using a homogenizer (ACE homogenizer-AM4 base with motor 250×245×380(II) mm, Tokyo, Japan) at full speed for 230-s intervals. The homogenate tissues were filtered though filter paper Whatman No 1 using vacuum. This extraction was repeated three times. The total soluble solid of flow through were determined by a hand refractometer. Results were expressed as % (Javanmardi and Kubota, 2006). Dry matter of the tuber was determined by drying in a hot-air oven at 105 ^{o}C for 3 h or until constant weight. Dry matter was expressed as a percentage of total weight (AOAC Method 925.10, 2005). Each sampling was performed in duplicate.

3.2 Effects of low temperature on inulin in Jerusalem artichoke tubers

3.2.1 Storage conditions

CN52867 cultivar of Jerusalem artichoke tubers (Pimsaen et al., 2010), were grown on Khon Kaen University Farm, Khon Kaen, Thailand from September to January (2010-2011). Jerusalem artichoke tubers were harvested at 50 days after flowering, then soaked in 0.038 M sodium hypochlorite for 30 min to eliminate soil and reduce microorganisms. Jerusalem artichoke tubers (CN 52867) (sample used in this study) are shown in Figure 3.1 They were then packed in sealed polyethylene bags and kept at different temperatures: -18, 0, 5, 10, 15, and 25 ^{o}C. The tubers were stored for 0, 5, 10, 15, 20, 25, and 30 days. Some samples were selected for fresh tuber analysis from each pack, then freeze-dried for preservation to prevent them from brown rot. Jerusalem artichoke powder was obtained as described in 3.1.1. The prepared samples were stored in a dry container at about -20 ^{o}C for further use.

Figure 3.1 Jerusalem artichoke tubers (CN 52867)/Sample used in this study.

3.2.2 Determination of inulin profiles, inulin content, soluble sugars, and 1-FFT activity

Tubers were analyzed for inulin profile, inulin content, soluble sugars content (fructose, glucose, sucrose), kestose, nystose, and 1-FFT activity in the same maner as in 3.1.2-3.1.6

3.2.3 Assay of inulin hydrolase (lnH) activities

lnH activity was analyzed as described by Ishiguro et al. (2010). One unit of lnH activity is defined as the amount of enzyme which hydrolyzes 1 nmol of inulin in 1 min. A mixture solution of enzymes (100 µL), 0.5% inulin solution (200 µL), and McIlvaine buffer (pH 5.5, 100 µL) was incubated at 30 ^{o}C for 5 h. The reaction was stopped by heating in boiling water for 10 min. lnH activity was calculated from the amount of fructose released as analyzed by HPAEC-PAD in the same maner as in 3.1.3.

3.3 Effects of CO_2 concentrations on inulin in Jerusalem artichoke tubers

3.3.1 Sample preparation

CN52867 cultivar of Jerusalem artichoke tubers were grown and harvested on the farm of Suranaree University of Technology from September to February (2011-2012). Jerusalem artichoke tubers were harvested at 50 days after flowering, then soaked in 0.038 M sodium hypochlorite for 30 min to eliminate soil and reduce microorganisms. The packaging configuration was 150 g of tubers packed in sealed high density polyethylene bags (HDPE) (Size 7×11 inch, 0.14 mm thickness). For CO_2 effects, CO_2 at 0, 5, 10, 15, and 20% were investigated with the fixed

percentage of oxygen (21%) whereas nitrogen was varied to make up the rest to 100% using a Gas mixer (WITT-Gasetechnik Gasetechnik Gasetechnik GmbHCo&KGD-58454, Germany) and the gas ratio was checked by oxybaby as described in Table 3.1

Table 3.1 Modification of gases in Jerusalem artichoke tubers packaging.

Treatment	Gas (%)		
	CO_2	O_2	N_2
1	0	21	79
2	5	21	74
3	10	21	69
4	15	21	64
5	20	21	59

The tubers were stored for 0, 5, 10, 15, 20, 25, and 30 days at 10 ^{o}C. Some samples were selected for analysis on the harvest date, then freeze dried for preservation to prevent them from brown rot. Jerusalem artichoke powder was obtained by peeling, slicing, freeze drying, and milling before the whole sample was passed through a 0.15 mm sieve (150 Micron). The prepared samples were stored in a dry container at -18 ^{o}C for further use.

3.3.2 Determination of inulin profiles, inulin content, soluble sugars, 1-FFT activity, and lnH activity

Sample were analysed for inulin profiles, inulin content, soluble sugars, 1-FFT activity, and lnH activity in the same maner as described in 3.1.2-3.1.6 and 3.2.3, respectively.

3.3.3 Correlations between CO_2 in the package and inulin content in Jerusalem artichoke tubers

Experiments were conducted according to a completely randomized design and correlations between CO_2 in the package and inulin content in the Jerusalem artichokes were calculated using the SPSS for Windows software version 16.

3.4 Statistical analysis

All statistical analyses were performed, using the SPSS for Windows software version 16. Data was analyzed by analysis of variances (ANOVA) one-way fixed factor. Duncan's multiple range test was calculated for mean comparisons at a significance level of $P<0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Influence of harvesting periods on inulin in Jerusalem artichoke tubers

4.1.1 Inulin profiles

The harvesting periods of Jerusalem artichokes also affected the quality of inulin (Figure 4.1). Inulin profiles were significantly different during the period of 30 to 70 days after flowering. Low DP 3-20 increased, while high DP 21-60 component decreased during 30-70 days. This suggested that the inulin profile changes with maturity. The decrease in DP 21-60 was probably due to depolymerization of fructan by fructan 1-exohydrolase (1-FEH) (Edelman and Jefford, 1968). It has been shown that 1-FEH exhibits a high affinity for fructan with a DP up to 30 (Bonnett and Simpson, 1993). Chekroun et al. (1994), using HPLC and TLC techniques, found that only late maturity tubers had the maximum content of polyfructan. The drying period of Jerusalem artichoke leaves and stems is accompanied by a small increase in reducing sugar, because of depolymerization of high molecular weight carbohydrate molecules (Schorr-Galindo and Guirand, 1997). In this study, tubers at 50 days after flowering contained high-DP fructan, compared to 60 and 70 days after flowering suggesting that inulin depolymerization occurred (Figure 4.1). Inulin DP profile of tubers harvested after 50 days of flowering presented in Figure 4.2. DP of inulin covered a wide range from DP2- DP 50 and over.

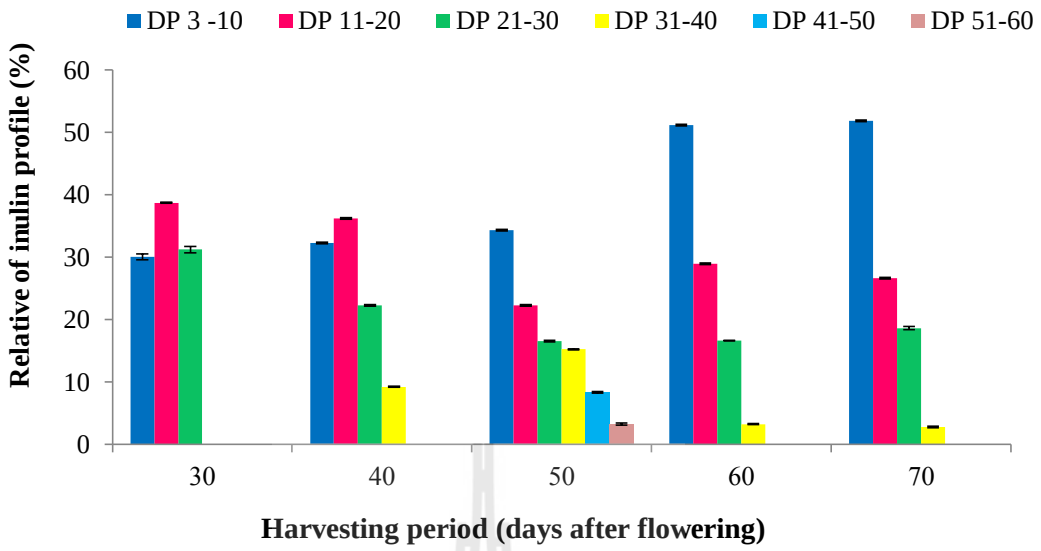

Figure 4.1 Relative percentages of inulin profiles in Jerusalem artichoke tubers at different harvesting periods.

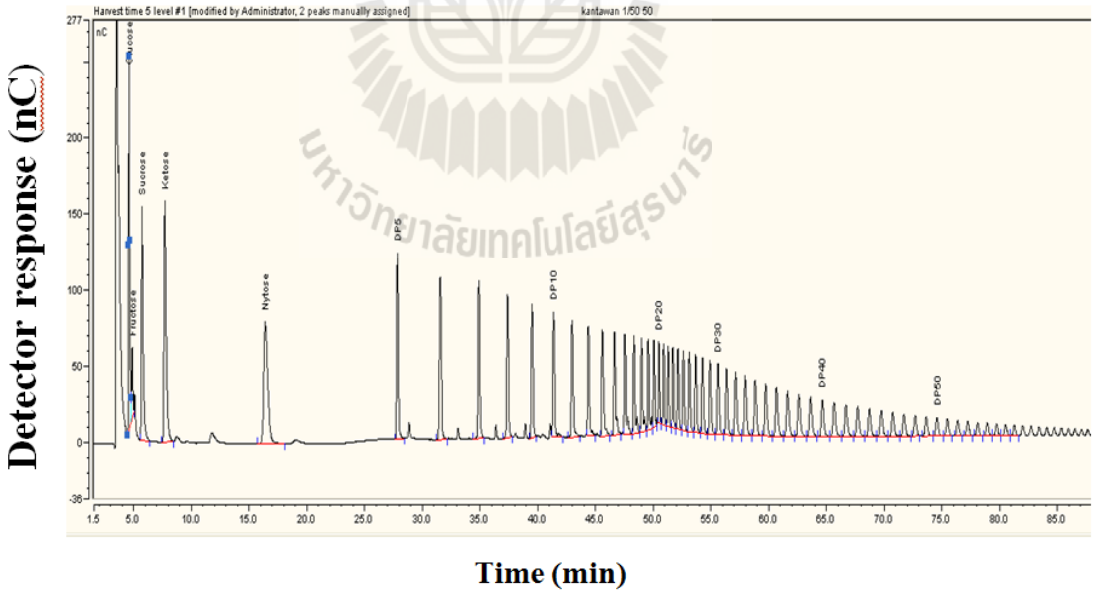

Figure 4.2 HPAEC-PAD Chromatograms of inulin profiles in Jerusalem artichoke tubers were harvested after 50 days of flowering.

4.1.2 Inulin contents

Inulin contents were significantly different during the period of 30 to 70 days after flowering ($P<0.05$) (Figure 4.3). Harvesting periods at 50 days had the highest inulin content while the content decreased at 60 and 70 days after flowering. This would be the result of synthesis or polymerization of fructan by 1-FEH. Tubers harvested at 50 days after flowering contained relatively high inulin content compared to 60 and 70 days after flowering, though there were no significant differences inulin depolymerization has been reported to be the act of 1-FEH in Jerusalem artichoke tubers (Rutherford and Deacon, 1972; Smith, 1976).

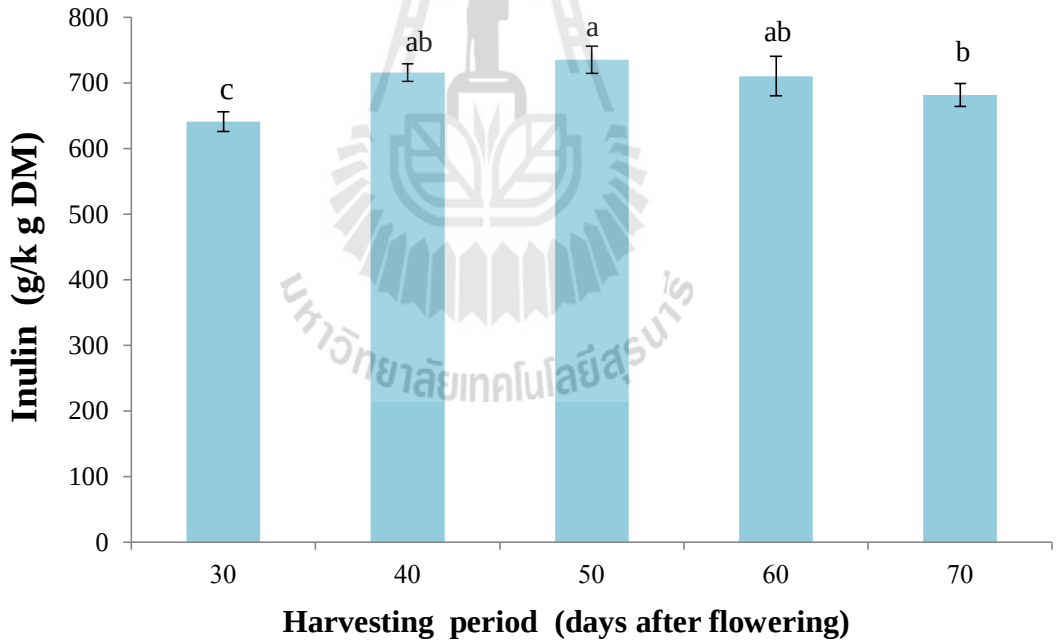

Figure 4.3 Changes of inulin content in Jerusalem artichoke tubers at different harvesting periods.

4.1.3 Activity of fructan:fructan 1- fructosly transferase (1-FFT)

Activities of fructan:fructan 1-fructosly transferase (1-FFT) were significantly different during harvesting at 30 to 70 days after flowering. Harvest periods 30 days after flowering had the highest activity of 1-FFT. The activity of 1-FFT decreased progressively in pseudo-linear regression after long flowering period. 1-FFT decreased from 79.9 to 28.03 U g^{-1} DM at 30 and 70 days after flowering, (Figure 4.4) respectively. In comparison of the activity of 1-FFT with inulin content it showed that 1-FFT had been synthesized and then inulin accumulation was catalyzed. Fructan biosyntheses take place by sucrose:sucrose 1-fructosyl transferase (1-SST) and 1-FFT activities in vacuole. Sucrose within the cytosol moves into the vacuole where fructose is removed by 1-SST and attached to a second sucrose molecule forming 1-ketose. The fructose is then removed by 1-FFT and attached to the growing fructan chain. Through repeat cycles, individual fructose molecules are progressively added, with the final length of the chain. Key enzymes in the synthesis of inulin in vacuole are 1-SST and 1-FFT (Figure 4.5).

Edeman and Jefford (1968) indicated that fructan synthesis was controlled by SST and 1-FFT. The DP of fructan produced by the plant depended mainly on the enzymatic activity of 1-FFT, although this enzyme could catalyze some chain-elongation reaction, ranging from the ability to synthesis small amounts of short-chain oligomers to the extensive synthesis of high-DP fructan (Van Laere and Van den Ende, 2002). Furthermore, the action of 1-SST and 1-FFT results in the formation of a mixture of fructan with different chain lengths. Starting from sucrose, 1-SST produces 1-ketose, which can be elongated by 1-FFT, resulting in the formation of FOS (Vijn and Smeeken, 1999). In Jerusalem artichoke tubers 1-SST and 1-FFT are active during fructan accumulation (Pollock, 1986).

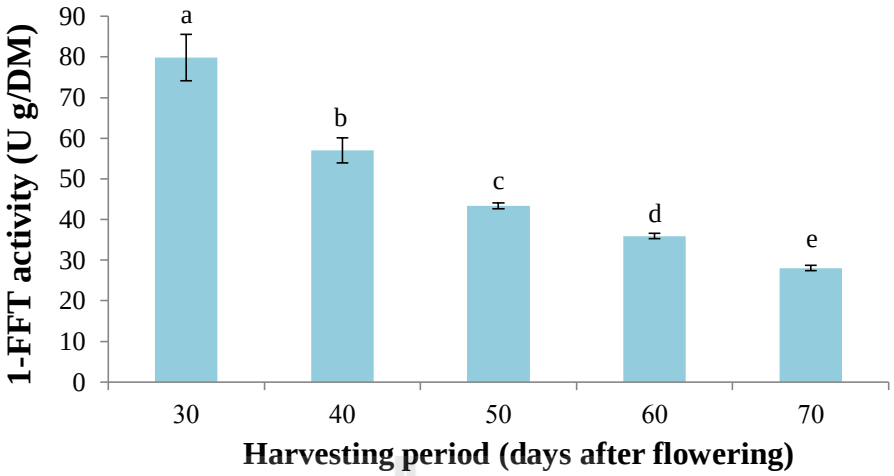

Figure 4.4 Changes in activity of 1-FFT in Jerusalem artichoke tubers at different harvesting periods.

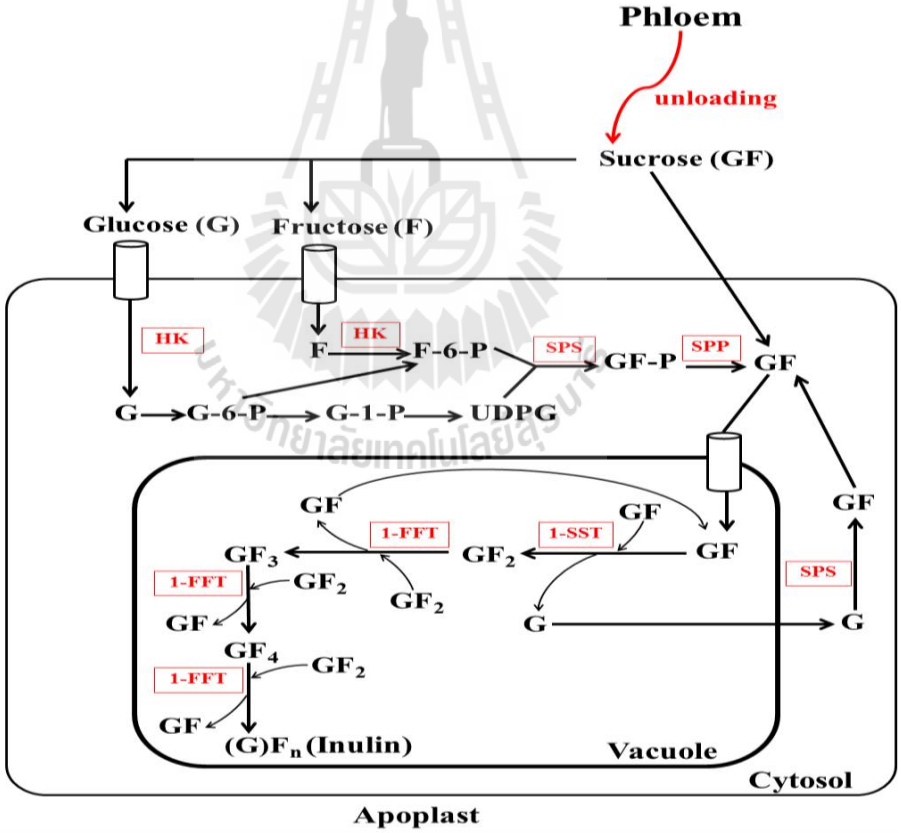

Figure 4.5 Fructan biosynthesis by 1-SST and 1-FFT in vacuole. (HK = hexxokinase; sps = sucrose phosphate synthase; spp = sucrose phosphate phosphatase) (Van Laere and Van den Ende, 2013)

4.1.4 Soluble sugars, kestose, and nystose content

Soluble sugars contents and kestose were significantly different during the period 30 to 70 days while nystose was not significantly different (P<0.05) (Figures 4.6-4.7). In the present study, the free glucose decreased, while free fructose and sucrose increased. The decrease in free glucose would be due to its utilization in the respiration process and metabolic activity (Jaime et al., 2001). The initial step of inulin synthesis is the conversion of two molecules of sucrose into one molecule of 1-kestose and glucose by 1-SST. In the next step, 1-kestose is converted to higher DP inulin by 1-FFT. Thus 1-kestose is considered to be an important intermediate for the production of inulin (Fukai et al., 1997). 1-SST is the first step of fructan synthesis in growing tuber, using sucrose as the primary source of fructosyl donor and releasing free glucose. Glucose usually appears in growing tubers and decreases to a very low level in mature tubers.

The increased levels of free fructose and sucrose after 50 days harvesting periods can be attributed to the hydrolysis of inulin. Jerusalem artichoke uses free fructose and sucrose for metabolic activity as substitutes for glucose. The soluble sugar changes can be attributed to the depolymerization process modulated by two enzymes, 1-FEH and 1-FFT, which catalyzes the release of free fructose, which in turn will be used for the re-synthesis of sucrose for export (Itaya et al., 2002).

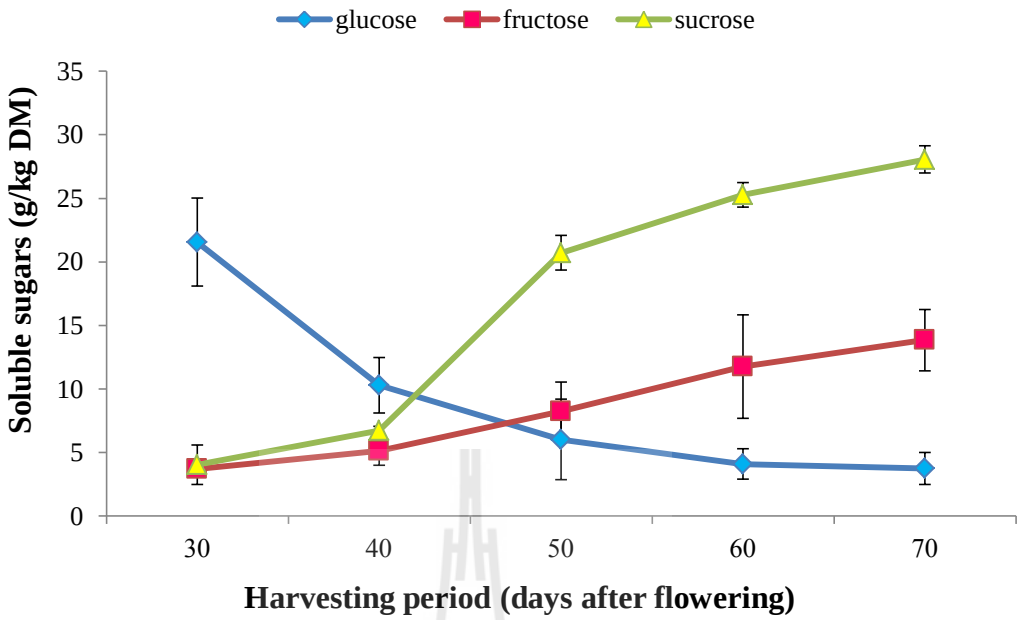

Figure 4.6 Changes of glucose, fructose, and sucrose contents in Jerusalem artichoke tubers at different harvesting periods.

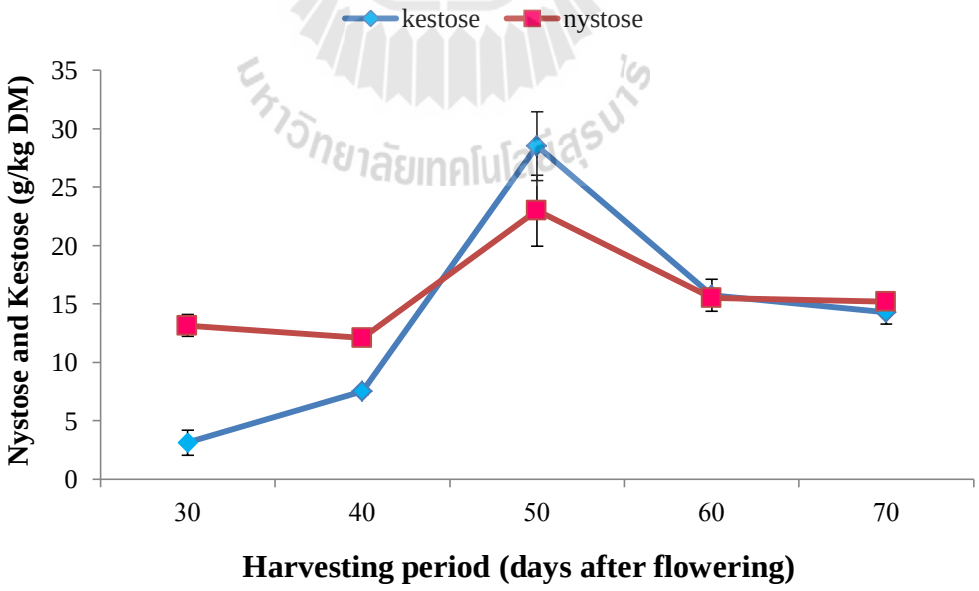

Figure 4.7 Changes of nystose and kestose contents in Jerusalem artichoke tubers at different harvesting periods.

4.1.5 Inulin content and yield of Jerusalem artichoke tubers

The edible part of Jerusalem artichoke represents about 65% of the total Jerusalem artichoke weight. Tuber weight, tuber yield and inulin yield were significantly different during the period of 30 to 70 days after flowering. Harvesting periods at 60 and 70 days gave the highest tuber weight and tuber yield. When the inulin content was compared with the tuber yield as dry matter per rai, showed that harvesting periods at 60 days after flowering had the highest amount of inulin content per rai (Table 4.1). From the production section, the tubers should be harvested at 60 days after flowering because the highest inulin yield per rai would be obtained.

Table 4.1 Influence of harvesting periods on tuber weight, tuber yield, and inulin yield in Jerusalem artichoke tubers.

Weight	Harvesting periods (days after flowering)				
	30	**40**	**50**	**60**	**70**
Tuber weight (g/plant)	163^{d}	465^{c}	1,037^{b}	1,660^{a}	1,688^{a}
Tuber yield (%FW)	55^{c}	58^{b}	65^{a}	65^{a}	65^{a}
Tuber yield (%DM)	17.37^{e}	20.54^{d}	25.36^{a}	24.71^{b}	23.61^{c}
Inulin yield (kg/rai)	107^{e}	342^{d}	783^{c}	1,212^{a}	1,182^{b}

Values with the same letter in each row are not significantly different at $P<0.05$.

4.1.6 Total soluble solid and dry matter

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 late maturity of 70 days after flowering had a spongy texture at the base of the tuber. Total soluble solids and dry matter were

significantly different within the harvesting periods (P<0.05). Tubers harvested at 50 days after flowering had the highest quantity of total soluble solids and dry matter. The dry matter of tubers increased from 30 to 50 days after flowering, while the dry matter of tubers decreased in harvesting periods 60 to 70 days after flowering (Table 4.2). This meaned that nature tubers accumulated relatively more water comparing with inulin content as tuber weight increases.

Table 4.2 Changes in the total amounts of soluble solids and dry matter in Jerusalem artichoke tubers with different harvesting periods.

Harvesting periods (days after flowering)	Total soluble solids (^{o}Brix)	Dry matter (%)
30	14±0.57^{e}	17.37±0.39^{e}
40	18±0.57^{d}	20.54±0.17^{d}
50	26±0.05^{a}	25.36±0.20^{a}
60	24±0.57^{b}	24.71±0.16^{b}
70	22±0.57^{c}	23.61±0.14^{c}

Values with the same letter in each column are not significantly different at P< 0.05.

4.2 Effects of low temperature on inulin in Jerusalem artichoke tubers

4.2.1 Inulin profiles

The temperature storage of Jerusalem artichokes also affected the quality of inulin (Figure 4.8). Inulin profiles were significantly different at different temperatures (P≤0.05). Storage of Jerusalem artichoke tubers at -18 ^{o}C could retard changes of inulin profile and therefore gave the highest DP>30 (12.13%). For storage at 0-25 ^{o}C, changes in DP>21 were very high and almost the same (no significant differences), resulting in higher DP 3-10. The decrease in DP 21-60 would be the result of depolymerization of fructan by 1-FEH (Edelman and Jefford, 1968). It was shown

that 1-FEH exhibits a high affinity for fructan with a DP up to 30 (Bonnett and Simpson, 1993).

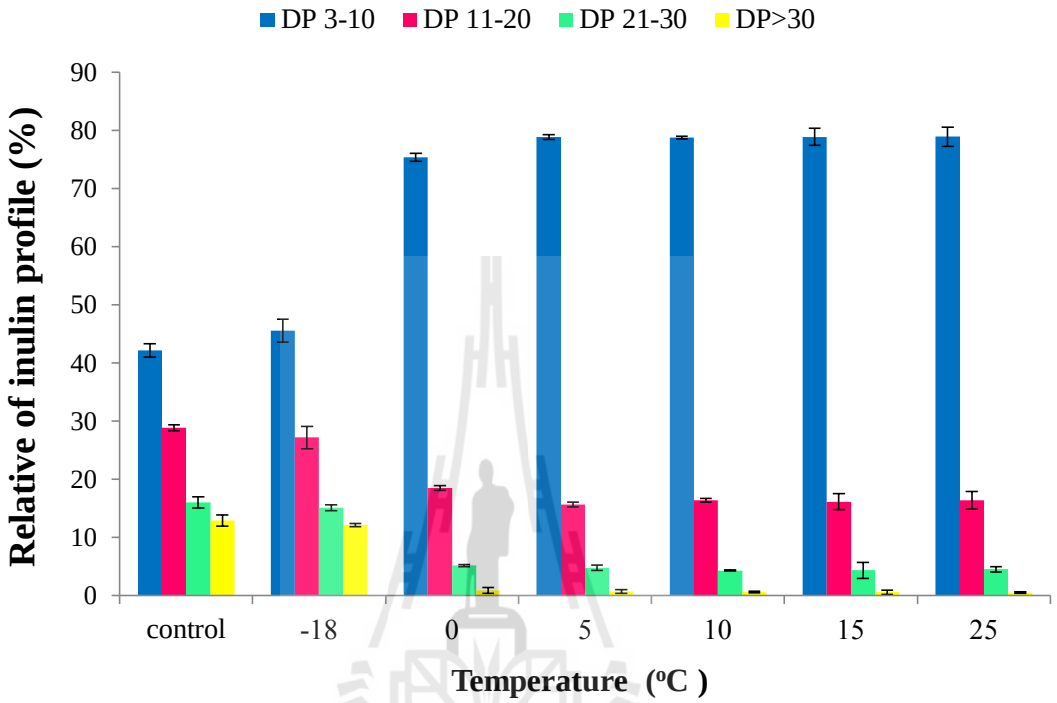

Figure 4.8 Relative % of inulin profiles in Jerusalem artichoke tubers stored at different temperatures for 30 days.

4.2.2 Inulin contents

At the harvest date of 50 days after flowering, inulin content in the Jerusalem artichoke tubers averaged 734.16 g/kg DM (Figure 4.9). Inulin contents were significantly different at different storage temperatures over the 30 day period ($P \leq 0.05$). Jerusalem artichoke tubers stored at -18 ^{o}C had the highest content of inulin (663.20 g/kg) although inulin content least decreased after 5 days of storage. For storage at 0, 5, 10, 15, and 25 ^{o}C, inulin content decreased gradually after 5 days with greater loss at higher storage temperatures ($P \leq 0.05$). The final inulin content was

625.07, 600.84, 590.05, 580.52, and 548.86 g/kg DM, at 0 to 25 ^{o}C respectively. This would be the result of depolymerization of fructan by 1-FEH. Rutherford and Weston (2001) showed that cold storage of chicory roots, Jerusalem artichokes, and dandelion caused a breakdown of inulin and high-DP fructosyl polymers to lower DP. These changes were nearly complete within the first 6 weeks of cold storage. The inulin DP changes of Jerusalem artichoke were more pronounced at higher storage temperatures. Benkeblia et al. (2002) showed that the hydrolysis of fructo-oligosaccharides was higher at 20 ^{o}C than at 10 ^{o}C in onion bulbs stored for 6 months.

Changes of inulin is expected since it has been reported that enzymes having 1-FEH activity are responsible for inulin degradation in these tubers (Rutherford and Deacon, 1972; Smith, 1976). From the present study, Jerusalem artichoke tubers stored at -18 ^{o}C would be preferable method for storage to maintain high inulin before processing.

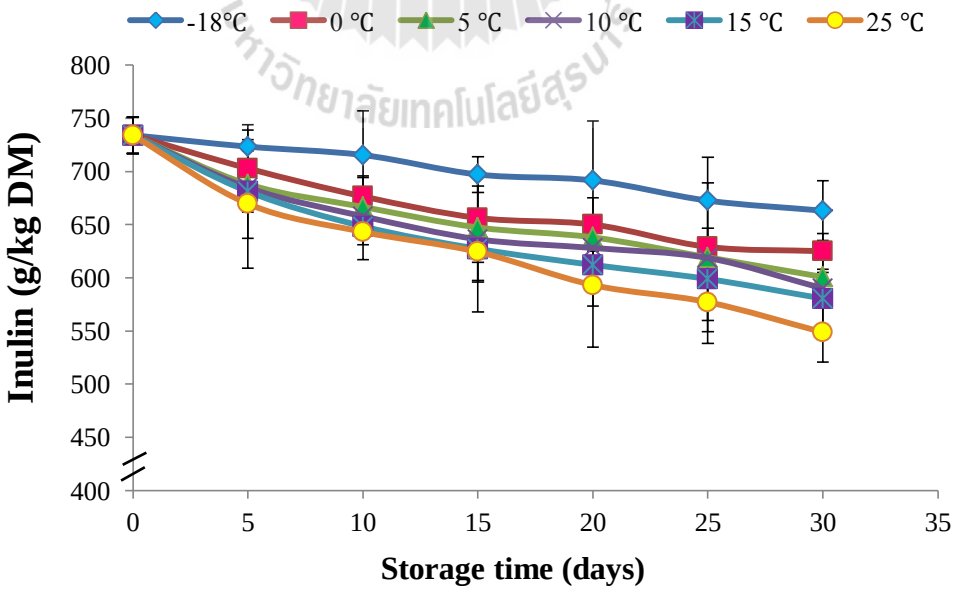

Figure 4.9 Changes of inulin contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

4.2.3 Activity of fructan:fructan 1-fructosyl transferase (1-FFT)

Activities of 1-FFT were significantly different ($P \leq 0.05$) at storage temperatures of -18, 0, 5, 10, 15, and 25 ^{o}C for 30 days (Figure 4.10). Initial 1-FFT activity at harvest time was high and then decreased significantly ($P \leq 0.05$) during storage at all conditions over 30 day. However, storage temperature at -18 ^{o}C gave the highest 1-FFT activity compared to higher temperature storage conditions. The lowest 1-FFT activity was at the highest storage temperature (25 ^{o}C). The activity of 1-FFT decreased progressively in pseudo-linear regression after 5 days of storage time. 1-FFT decreased from 44.37 to 22.39 and to 11.45 U g^{-1} DM at -18 ^{o}C and 25 ^{o}C, respectively.

Edeman and Jefford (1968) showed that fructan synthesis was controlled by SST and 1-FFT. The DP of fructan produced by the plant depended mainly on the enzymatic activity of 1-FFT, although this enzyme was able to catalyze some chain-elongation reaction, ranging from the ability to synthesize small amounts of short-chain oligomers to the extensive synthesis of high-DP fructans (Van Laere and Van den Ende, 2002). Furthermore, the action of 1-SST and 1-FFT resulted in the formation of a mixture of fructans with different chain lengths. Starting from sucrose, 1-SST would produce 1-ketose which is catalyzed by 1-FFT, resulting in the formation of fructooligosaccharide (FOS) (Vijn and Smeeken, 1999). In Jerusalem artichoke tubers, 1-SST and 1-FFT were active during fructan accumulation (Pollock, 1986). 1-FFT was present during both inulin synthesis and depolymerization, although its activity decreased during tuber storage (Edeman and Jefford, 1968). During synthesis at appropriate conditions, 1-FFT would catalyze chain elongation. However, during shortage of sucrose depolymerization would be catalyzed by 1-FFT via moving fructosyls from the longer-chain-length polymers to obtain free sucrose (Luscher et al., 1993b). Similar to the results of the present study, Ishiguro et al. (2010) reported the

variability of 1-FFT in burdock roots stored at 0, 15, and 25 ^{o}C, and the activity of 1-FFT decreased progressively in a pseudo-linear regression during storage.

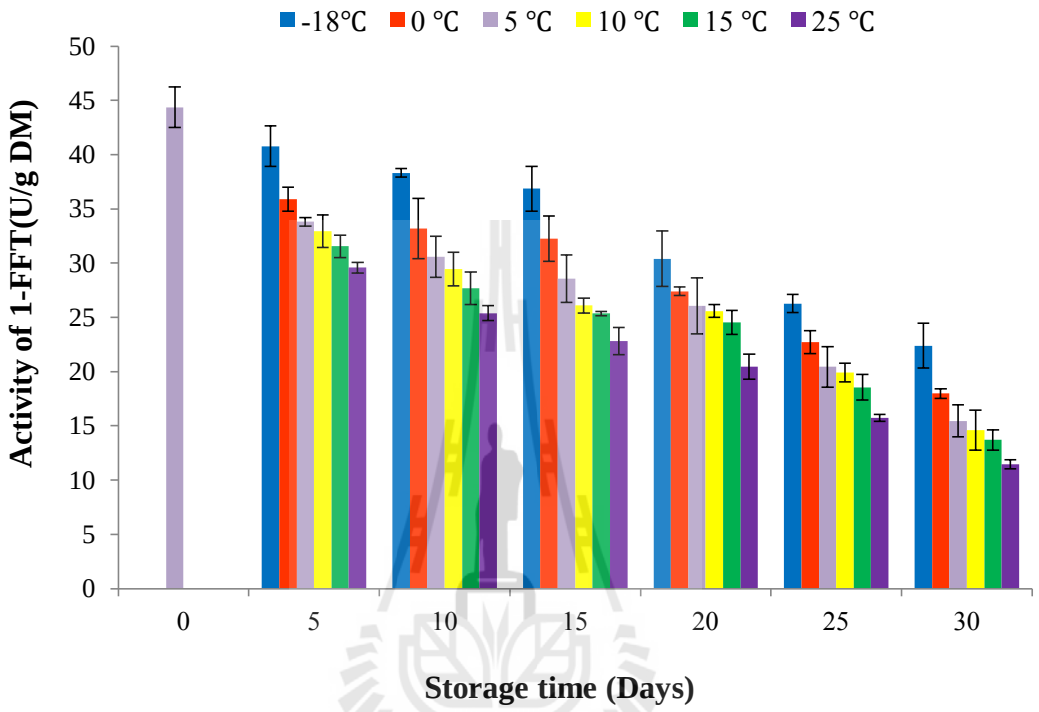

Figure 4.10 Changes in activity of fructan:fructan 1- fructosyl transferase in Jerusalem artichoke tubers stored at different temperatures for 30 days.

4.2.4 Activity of inulin hydrolase (lnH)

The activities of inulin hydrolase (lnH) were significantly different ($P \leq 0.05$) storage at -18, 0, 5, 10, 15, and 25 ^{o}C for 30 days (Figure 4.11). At -18 ^{o}C , lnH activity steadily decreased over the 30 day period, unlike storage at 0, 5, 10, 15, and 25 ^{o}C where lnH activity increased over the first 15 days of storage and then decreased. Changes were significantly different after 5 days at these different storage

temperatures. The activity of lnH decreased over the 30 day storage from 15.6 to 7.24 and to 15.40 U g^{-1} DW at -18 and 25^{o}C, respectively.

InH catalyzes inulin breakdown through cleavage between the terminal fructosyl group and its adjacent fructosyl residue, under the assuming that it does not catalyze sucrose hydrolysis (Franck and De Leenheer, 2005). Lower activity of lnH at low temperature storage explains lesser inulin breakdown. Ishimaru et al. (2004) reported the activity of inulinase in burdock roots stored in modified atmosphere packaging at 2, 8, and 20 ^{o}C, increased sharply after one week storage regardless of temperature. Ishiguro et al. (2010) also reported that the activity of lnH in burdock roots decreased progressively at 0, 15 and 20 ^{o}C during storage with more significance at 15 and 20 ^{o}C.

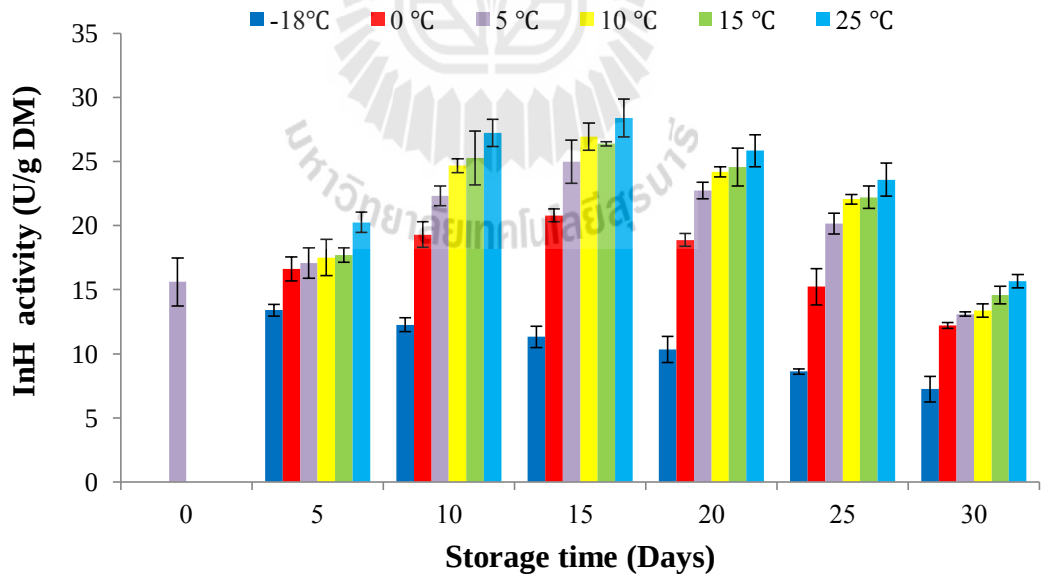

Figure 4.11 Changes in activity of inulin hydrolase in Jerusalem artichoke tubers stored at different temperatures for 30 days.

4.2.5 Soluble sugars, kestose, and nystose contents

Fructose,sucrose, kestose, and nystose were significantly different ($P \leq 0.05$) during storage at -18, 0, 5, 10, 15, and 25 ^{o}C for 30 days (Figures 4.12-4.16). Glucose content decreased over the storage period under the storage conditions indicated. On the other hand, fructose and sucrose contents continuously increased over this period. Kestose and nystose contents were increased after 5 days of storage. The decrease of glucose observed during storage was used in the respiration process and metabolic activities (Jaime et al., 2001). In the present study, increased levels of free fructose, sucrose, kestose, and nystose in stored Jerusalem artichoke tubers would be attributed to hydrolysis of inulin with no exception during cold storage (-18 ^{o}C), where hydrolysis still slowly proceeded.

Jerusalem artichoke tubers use free fructose and sucrose for metabolic activities as substitutes for glucose. As explained above, the changes in these soluble sugars, kestose, and nystose would be the result of the depolymerization process modulated by the two enzymes 1-FEH and 1-FFT (Itaya et al., 2002). When the Jerusalem artichoke tubers are dormant, 1-SST activity disappears and 1-FEH appears. This enzyme removes fructosyl residues from the stored fructan. During cold storage, 1-FEH and about 80% of the sucrose are localized in vacuoles (Gupta and Kaur, 2000). Claessen et al. (1990) purified a fructanexohydrolase (inulinase) from chicory roots stored at 2-3 ^{o}C, which later called 1-FEH, and found that the enzyme was slightly inhibited by sucrose. Therefore, in dormant tubers, 1-FEH activity would be mainly moderated by concentration of sucrose and storage at low temperatures (De Roover et al., 1999). This means that 1-FFT redistributes fructose residues from high DP inulin polymers to low DP oligomers by transferring fructosyl residues on the sucrose (formed from the fructose released by 1-FEH) without accumulation of free fructose.

According to Edelman and Jefford (1968), based primarily on studies of Jerusalem artichoke tubers, the initial step of inulin synthesis is the conversion of two molecules of sucrose into one molecule of 1-kestose and glucose by 1-SST. In the next step, 1-kestose is converted to higher DP inulin by1-FFT. Thus, 1-kestose is considered to be an important intermediate for the formation of inulin (Fukai et al., 1997). 1-SST catalyzes the first step of fructan synthesis in growing tubers, using sucrose as the primary source of the fructosyl donor and releasing free glucose. Glucose usually appears in growing tubers and decreases to a very low level in mature tubers.

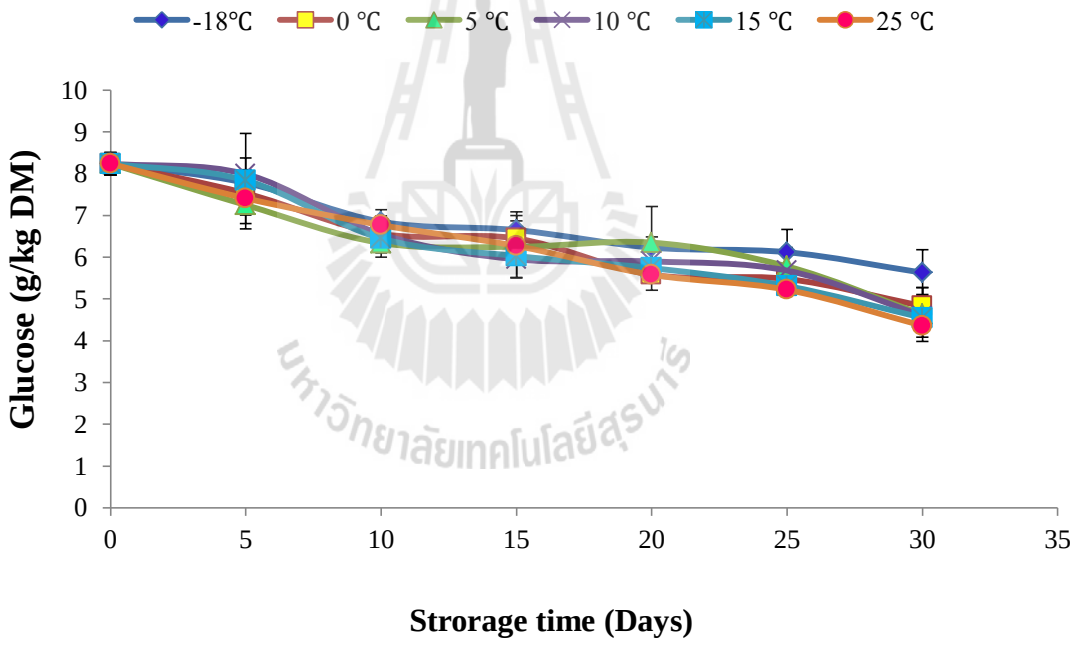

Figure 4.12 Changes in glucose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

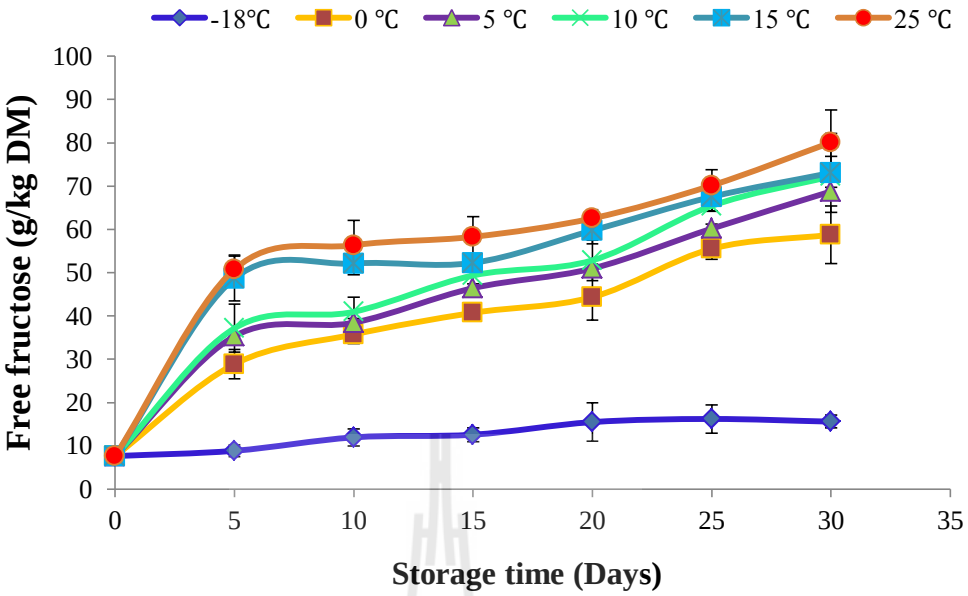

Figure 4.13 Changes in free fructose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

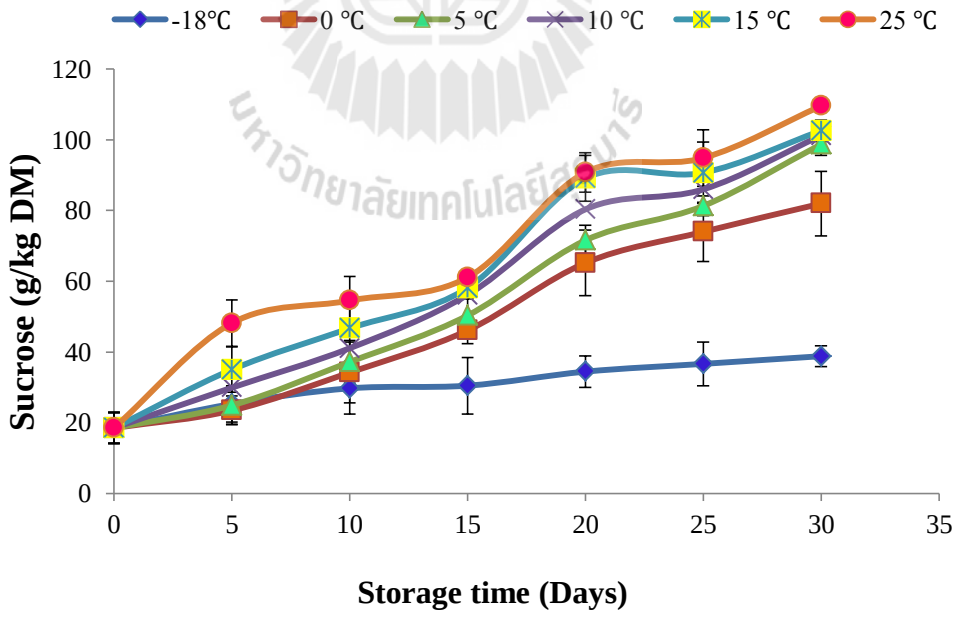

Figure 4.14 Changes in sucrose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

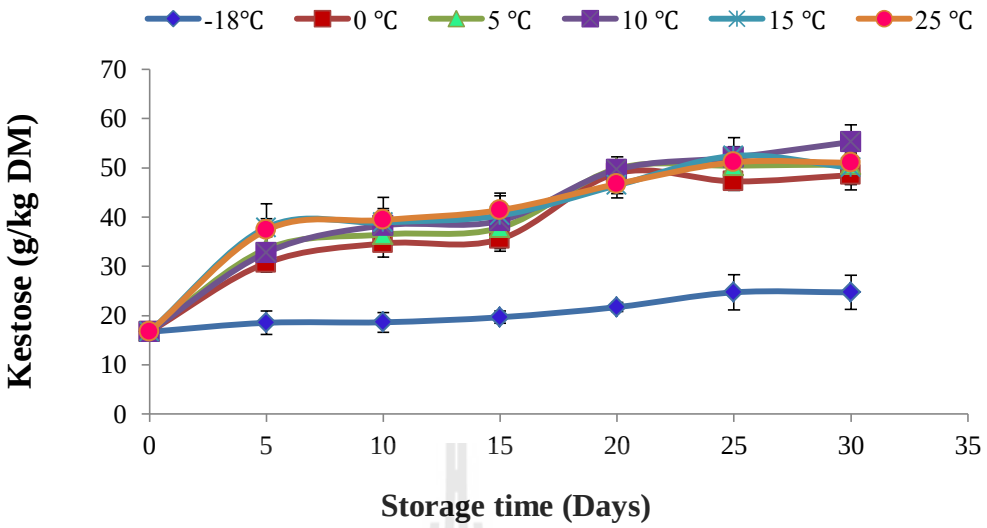

Figure 4.15 Changes in kestose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

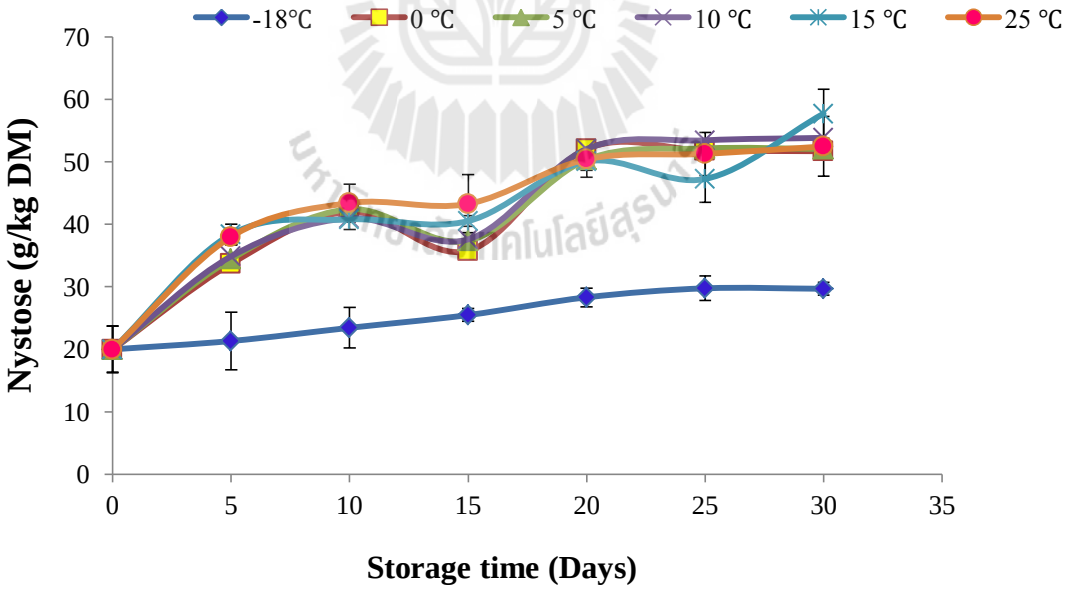

Figure 4.16 Changes in nystose contents in Jerusalem artichoke tubers stored at different temperatures for 30days.

4.3 Effects of CO_2 concentrations on inulin in Jerusalem artichoke tubers

4.3.1 Inulin profiles

CO_2 concentrate also affected the quality of inulin in Jerusalem artichoke tubers. After 30 days of storage inulin profiles were significantly different at differentce ($P \leq 0.05$) CO_2 concentrate (Figure 4.17). This suggests that the inulin profiles changes with the amount of CO_2 concentrate.

With MA CO_2 levels of 5% and higher, commodities have reduced respiration rates (Brecht, 1980; Kader, 1986). Kader (1986) pointed out that 10% CO_2 added to air reduced the respiratory rate of commodities to about the same extent as a 2% O_2 drop.

Elevated CO_2 contents in the microatmosphere around fruit and vegetables have an inhibitory effect on enzymes in the Krebs cycle and glycolysis pathways. Succinate has been found to accumulate in apple, apricots, carrots, peaches, pears, cherries and lettuce when CO_2 in the microatmosphere was elevated to 20% (Frenkel and Patterson, 1993; Monning, 1983). Such a situation implies that succinic dehydrogenase activity was inhibited.

Ranson et al. (1960) found that CO_2 inhibited succinic acid oxidase in isolated castor bean mitochondria. Shipway and Bramlage (1973) reported that, with isolated apple mitochondria exposed to a gas with 6% CO_2, malate oxidation was enhanced whereas the oxidation of citrate, α-ketoglutarate, succinate, fumurate, and pyruvate was reduced. They attributed these results to the structural and conformation changes in the mitochondria.

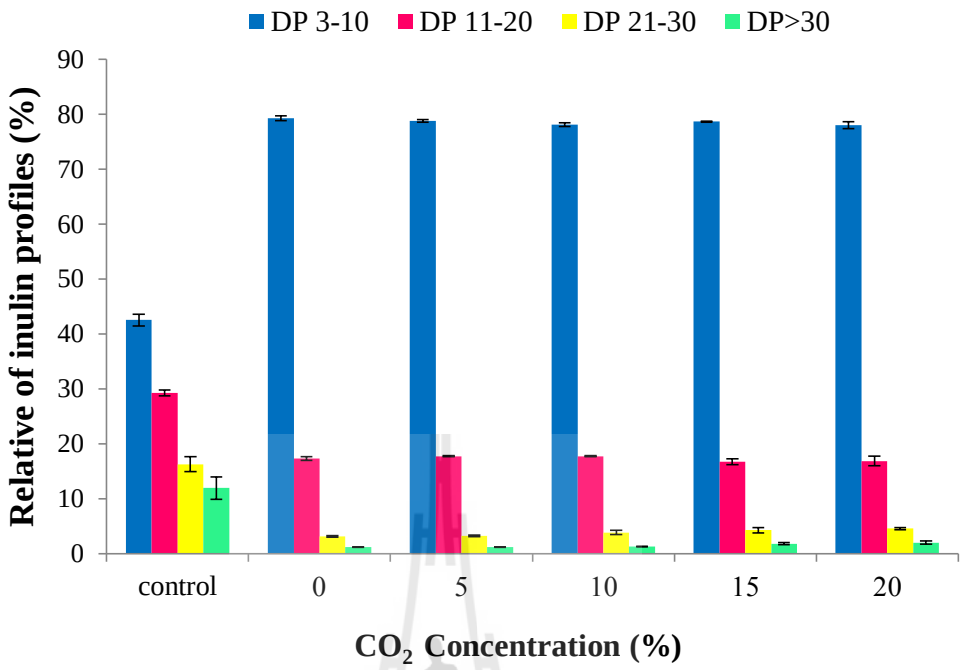

Figure 4.17 Relative percentages of inulin profile in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

4.3.2 Inulin contents

In fresh Jerusalem artichoke tubers after harvest, inulin content was about 723.69 g/kg DM. After 30 days storage, inulin content was significantly different ($P \leq 0.05$) at different concentrations of CO_2. The tubers stored at 20% CO_2 had the highest inulin content (625.06 g/kg). During storage at 0, 5, 10, 15, and 20% CO_2, inulin content significantly decreased ($P \leq 0.05$) after 5 days and the final inulin content was 545.17.09, 576.16, 597.21, 606.54, and 625.87 g/kg DM, respectively (Figure 4.18). This would be the result of depolymerization of fructan by 1-FEH, similar to the report of Rutherford and Weston (2001) on chicory roots, Jerusalem artichokes, and dandelion kept in cold storage. These changes were nearly complete within the first 6 weeks of cold storage. The inulin DP changes of Jerusalem artichoke were more

pronounced at higher storage temperatures (Modler et al., 2005). The hydrolysis of fructo-oligosaccharides was higher at 20 ^{o}C than at 10 ^{o}C in onion bulbs stored for 6 months (Benkeblia et al., 2002).

CO_2 treatment also prevented fruit softening and modified the activity and content of cell wall degrading enzymes (Cura et al., 1996). However, the levels and activities of polymerdegrading enzymes in cell walls are not always consistent with the rate of fruit softening. Nevertheless, 1-FEH would be responsible for inulin degradation in these tubers (Rutherford and Deacon, 1972; Smith, 1976).

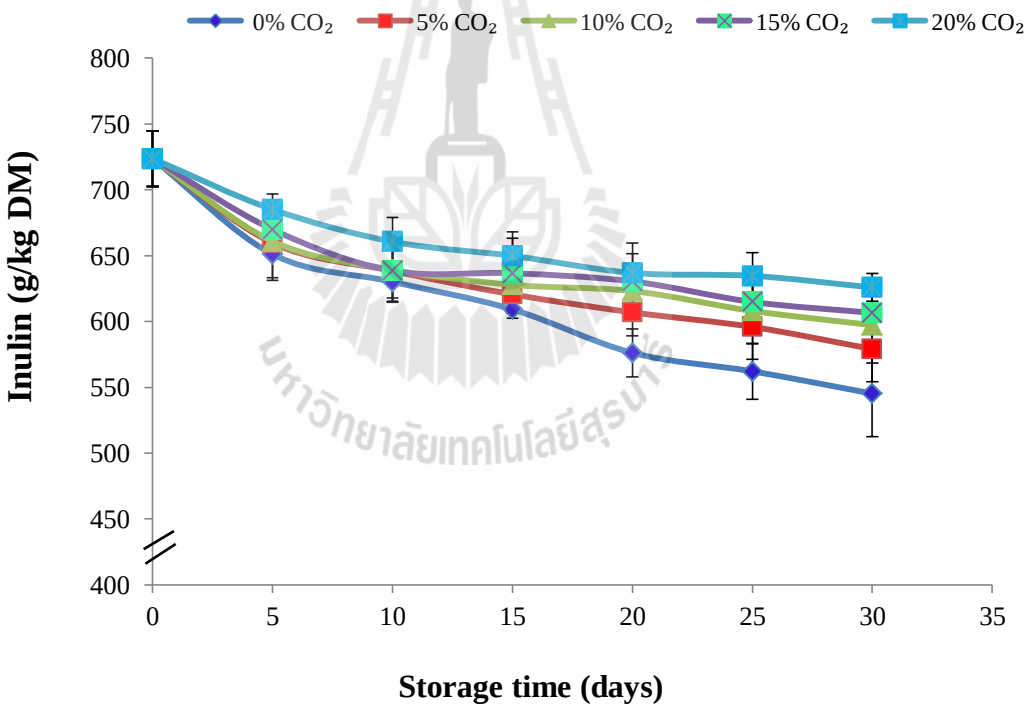

Figure 4.18 Changes of inulin contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

4.3.3 Activity of fructan:fructan 1-fructosyl transferase (1-FFT)

The activities of 1-FFT were not significantly different ($P \leq 0.05$) at different concentration of CO_2 storage over a 30 day period. The activity of 1-FFT decreased progressively in pseudo-linear regression after 5 days of storage, from 16.23 to 6.82 U g^{-1} DM and 16.23 to 6.15 U g^{-1} DM at 20% CO_2 and 0%, respectively (Figure 4.19). Edeman and Jefford (1968) indicated that fructan synthesis was controlled by 1-SST and 1-FFT. The DP of fructan produced in the plant depended mainly on the enzymatic activity of 1-FFT, although this enzyme can catalyze some chain-elongation reaction, ranging from the ability to synthesis small amounts of short-chain oligomers to the extensive synthesis of high-DP fructan (Van Laere and Van den Ende, 2002). Furthermore, the action of 1-SST and 1-FFT resulted in the formation of a mixture of fructan with different chain lengths. Starting from sucrose, 1-SST produced 1-ketose, then 1-ketose was elongated by 1-FFT, resulting in the formation of FOS (Vijn and Smeeken, 1999).

In Jerusalem artichoke tubers, 1-SST and 1-FFT were active during fructan accumulation (Pollock, 1986). 1-FFT was present during both inulin synthesis and depolymerization, although its activity decreases during tuber storage (Edeman and Jefford, 1968). During under synthetic conditions, 1-FFT catalyzed chain elongation, however, during depolymerization, when sucrose was not present, 1-FFT moved fructosyls from the longer-chain-length polymers to the shorter polymers (Luscher et al., 1993a). Similarly, Ishiguro et al. (2010) reported the variability of 1-FFT in burdock roots stored at 0, 15, and 25 ^{o}C, with progressive decreases in the activity in a pseudo-linear regression during storage. The effect of CO_2 depended on its dosage and environmental conditions such as temperature (Smith, 1992). High CO_2 concentrations at 20 ^{o}C would delay or inhibit ripening and senescence in fruit and vegetables.

Although most of the ripening-associated changes caused by high CO_2 would involve inhibition of ethylene production and action, high CO_2 perhaps regulated other development-dependent processes (Rothan et al., 1997). CO_2 treatment also prevented fruit softening and modified the activity and content of cell wall degrading enzymes (Cura et al., 1996). Kader (1986) pointed out that 10% CO_2 added to air reduced the respiratory rate of the commodity to about the same extent as 2% O_2 drop. The reduction of respiration rates was reported to associate with the inhibition of the activity of oxidases such as a polyphenol oxidase, ascorbic acid oxidase and glycolic acid oxidase (Burton, 1978).

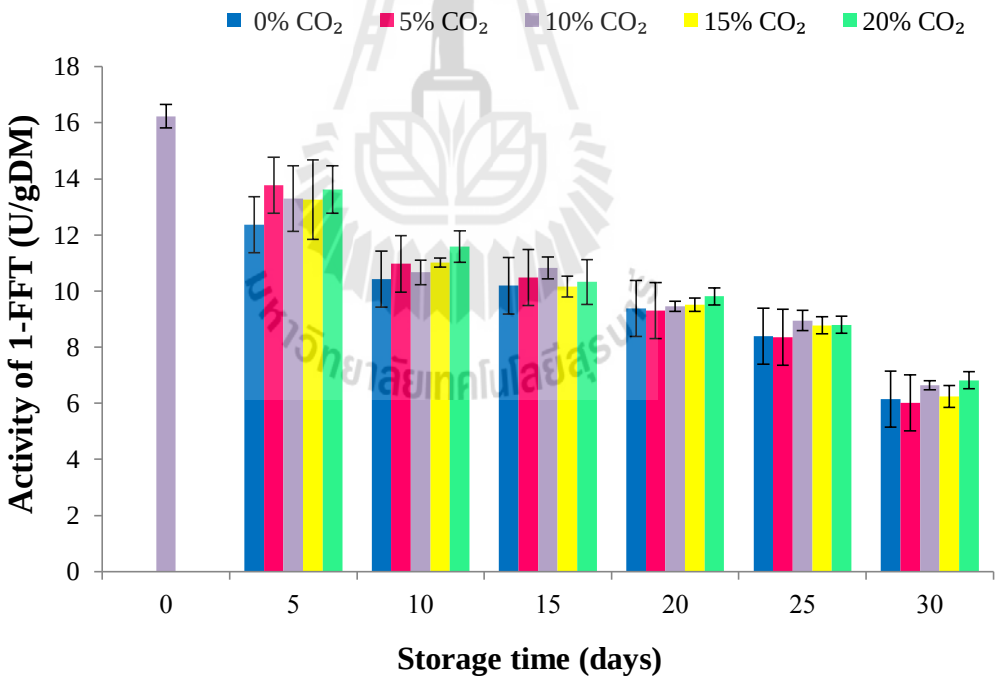

Figure 4.19 Changes in activity of 1-FFT in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

4.3.4 Activity of inulin hydrolase (lnH)

The activities of inulin hydrolase were significantly different ($P \leq 0.05$) at different concentrations of CO_2 over 30 days of storage. During storage at CO_2 concentrations of 0, 5, 10, 15, and 20%, lnH activity increased within 15 days and then gradually decreased. Changes at 10 days were not significant; however, after 10 days changes became significant. Storage for 15 days time maintained the highest activity of lnH. The activity of lnH increased from 13.51 to 17.54 and 13.51 to 23.84 U g^{-1} DW at 20% CO_2 and 0% CO_2, respectively, after 30 days (Figure 4.20). lnH would catalyzes inulin breakdown though cleavage between the terminal fructosyl group and its adjacent fructose residue, under the assumption that it did not catalyze sucrose hydrolysis (Franck and De Leenheer, 2005). Ishimaru et al. (2004) reported the activity of inulinase in burdock roots stored in modified atmosphere packaging at 2, 8, and 20 ^{o}C and noted that lnH increased sharply after one week of storage regardless of temperature; however, the increase was much higher at 2 ^{o}C. The decrease of lnH would lead to an accumulation of shorter fructans, which were in turn hydrolyzed successively by 1-ketose hydrolase (1-KH) and sucrose hydrolase (SH) to produce sucrose, glucose, and fructose. Ishiguro et al. (2010) reported the activity of lnH in burdock roots, lnH to be showed a different but more regular pattern with decreases progressive at 0, 15, and 20 ^{o}C. However, the decreases were more significant at 15 and 20 ^{o}C. Moreover, low temperatures induced fructans of hydrolyzes in onions (Darbyshire and Henry, 1978) and this action was the result of the activation of phosphorylases at low temperatures.

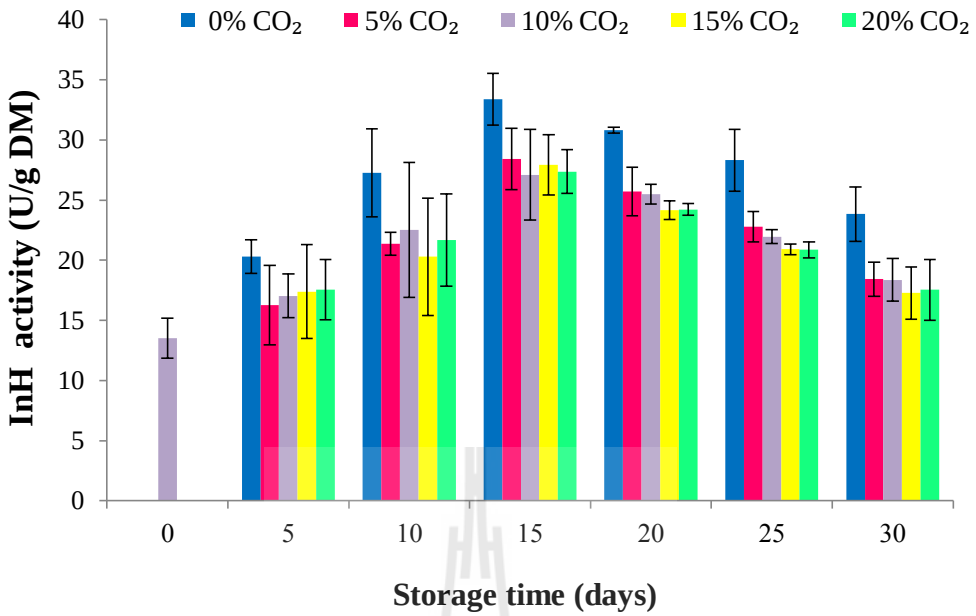

Figure 4.20 Changes in the activity of inulin hydrolase in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

4.3.5 Fructose, sucrose, kestose, and nystose contents

Fructose, sucrose, kestose, and nystose were significantly different ($P \leq 0.05$) at different concentrations of CO_2 over the 30 day storage. In the present study, the fructose content increased after 15 days of storage, then gradually decreased during prolonged storage, while sucrose, kestose, and nystose content increased during storage. The content of fructose increased from 8.37 to 11.27 and 8.37 to 22.63gkg^{-1} DM at 20% CO_2 and 0% CO_2, respectively, after 30 days. The content of sucrose increased from 10.38 to 50.99 and 10.38 to 77.29 gkg^{-1} DM at 20% CO_2 and 0% CO_2, respectively, after 30 days. The content of kestose increased from 17.23 to 43.20 and 17.23 to 35.59gkg^{-1} DM at 20% $CO_2$and 0% CO_2, respectively, after 30 days. The content of nystose increased from 20.26 to 44.53 and 20.60 to 39.92 g kg^{-1} DM at 20% CO_2 and 0% CO_2 ,respectively, after 30 days (Figures 4.21-4.24). Fructose decreases

after 15 days of storage would be explained by its utilization for the respiration process and by metabolic activities (Jaime et al., 2001). The increased levels of free fructose, sucrose, kestose, and nystose in stored Jerusalem artichoke tubers attributed to the hydrolysis of inulin. Jerusalem artichoke tubers would use free fructose and sucrose for metabolic activity as substitutes for glucose. The soluble sugar, kestose, and nystose changes would attribute to the depolymerization process modulated by two enzymes, 1-FEH and 1-FFT, catalyzing the release of free fructose in turn to be used for the re-synthesis of sucrose for export (Itaya et al., 2002). When the Jerusalem artichoke tubers are dormant, SST activity disappears and 1-FEH appears. This enzyme removes fructosyl residues from stored fructan. During cold storage, 1-FEH and about 80% of the sucrose are localized in vacuoles (Gupta and Kaur, 2000).

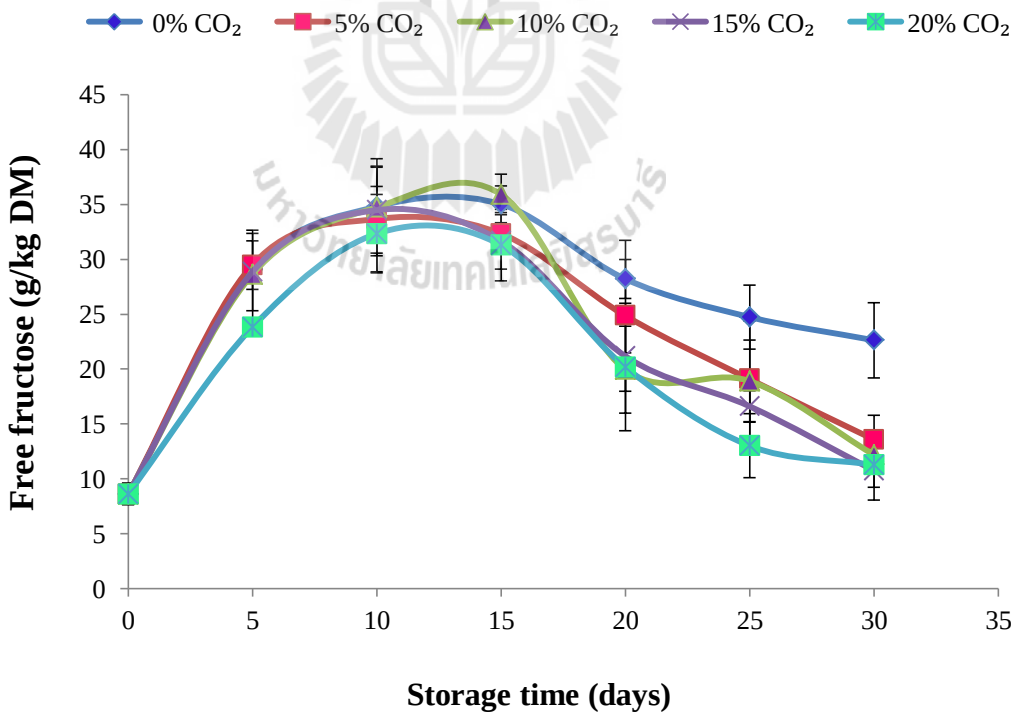

Figure 4.21 Changes of free fructose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

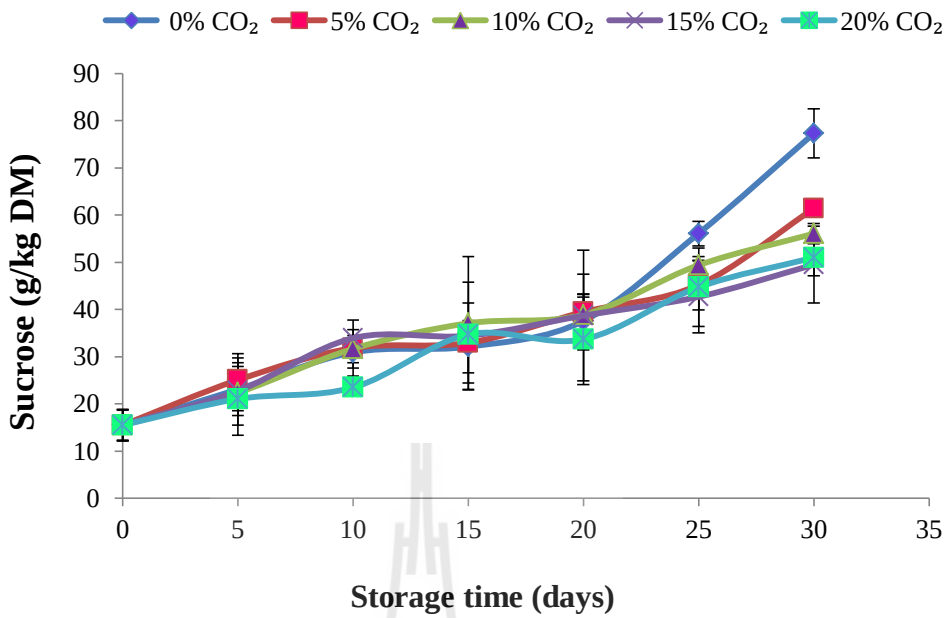

Figure 4.22 Changes of sucrose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

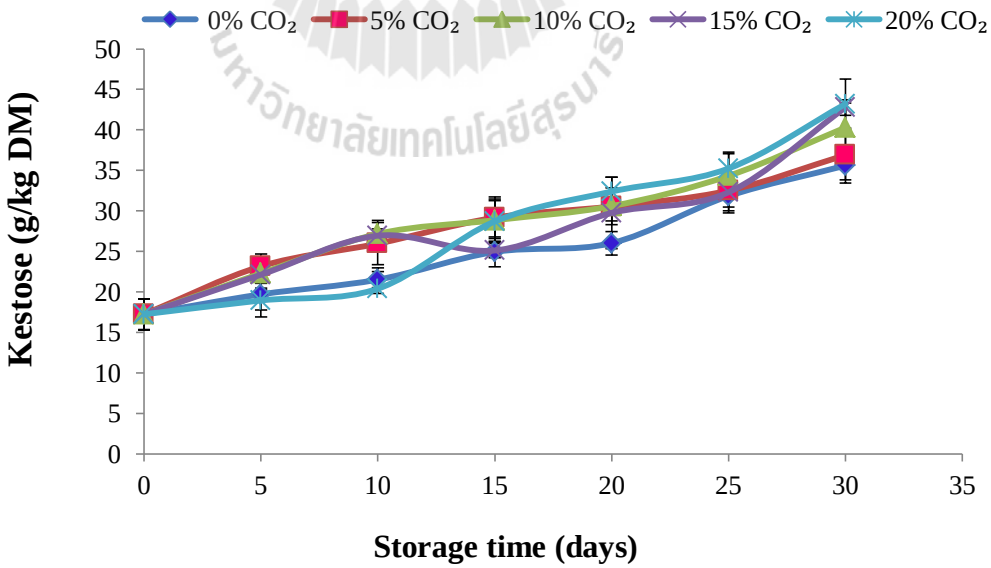

Figure 4.23 Changes of kestose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

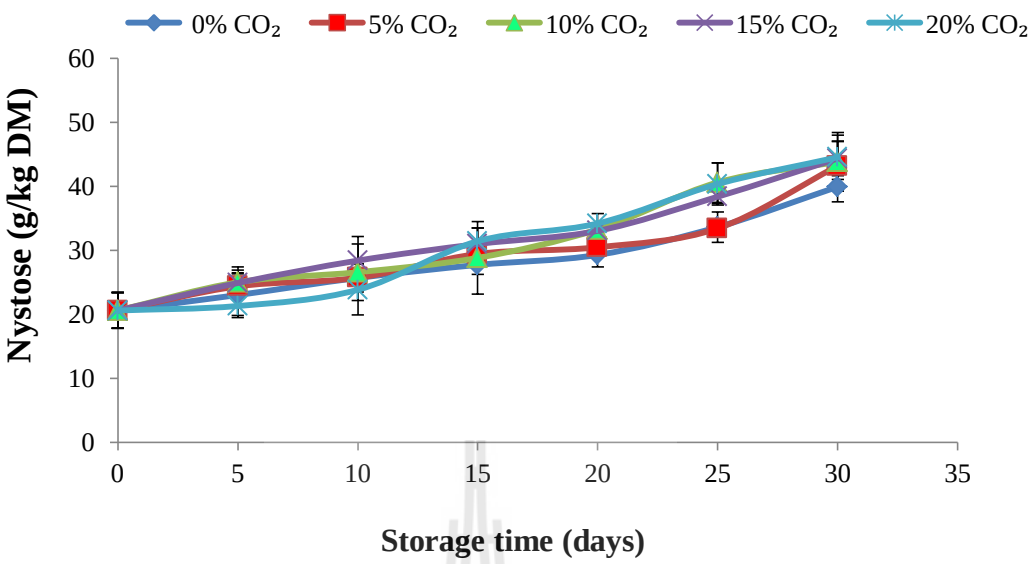

Figure 4.24 Changes of nystose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

4.3.6 Correlation between CO_2 concentration in the package and inulin contents in Jerusalem artichoke tubers.

CO_2 in the package changed during storage (Table 4.6). CO_2 in the package ranged from 0.05 to 20% in the first day of storage and after 30 days ranged from 7.37 to 13.33%. The inulin content in fresh Jerusalem artichoke tubers was 72.37% DM in the beginning of storage and after 30 days the inulin content ranged from 54.52 to 62.59% DM. There were linear relationships between CO_2 concentration in the package and the inulin contents in the Jerusalem artichoke tubers at 15, 20, 25, and 30 days of storage (Table 4.5; Figure 4.25).

Highly significant correlations were found between $\%CO_2$ in the package and the inulin contents in Jerusalem artichoke tubers at 15, 20, 25, and 30 days of storage ($R = 0.94$ $*$), ($R = 0.99$ $**$), ($R= 0.88$ $*$), and ($R = 0.96$ $**$), respectively. However, correlations between $\%CO_2$ in the package and the inulin contents in the

Jerusalem artichoke tubers at 5 and 10 days of storage were not significant (R = 0.87 NS) and (R = 0.94 NS), respectively. The data in this study showed that when $\%CO_2$ in the package was high it resulted in a slow decrease of inulin in the Jerusalem artichoke tubers. However, the amount of CO_2 at 30 days of storage was not more than 12.6%. Kader (1986) pointed out that 10% CO_2 added to air reduced the respiratory rate of the commodity to about the same extent as a 2% O_2 drop.

The reduction of respiration rates would attribute to the inhibition of the activity of oxidases such as a polyphenol oxidase, ascorbic acid oxidase and glycolic acid oxidase (Burton, 1978). Unsuitable MA would be responsible for inducing physiological damage, preventing wound healing, enhancing the senescence process and producing off-flavour compounds. Generally O_2 levels lower than about 1% in the microatmosphere will bring about anaerobic respiration (Boersig et al., 1988). CO_2 levels of 10% and above can inhibit the growth of spoilogens, but may cause physiological damage to CO_2 sensitive commodities (Goorani and Sommer, 1981; Kader et al., 1985).

Table 4.3 The Pearson correlation coefficient for CO_2 concentrations in the package correlated to the inulin contents in the Jerusalem artickoke tubers during storage.

Days	The Pearson correlation coefficient for CO_2 and inulin contents					
	5	**10**	**15**	**20**	**25**	**30**
R	0.87^{NS}	0.76^{NS}	0.94^{*}	0.99**	0.88*	0.96**

ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01.

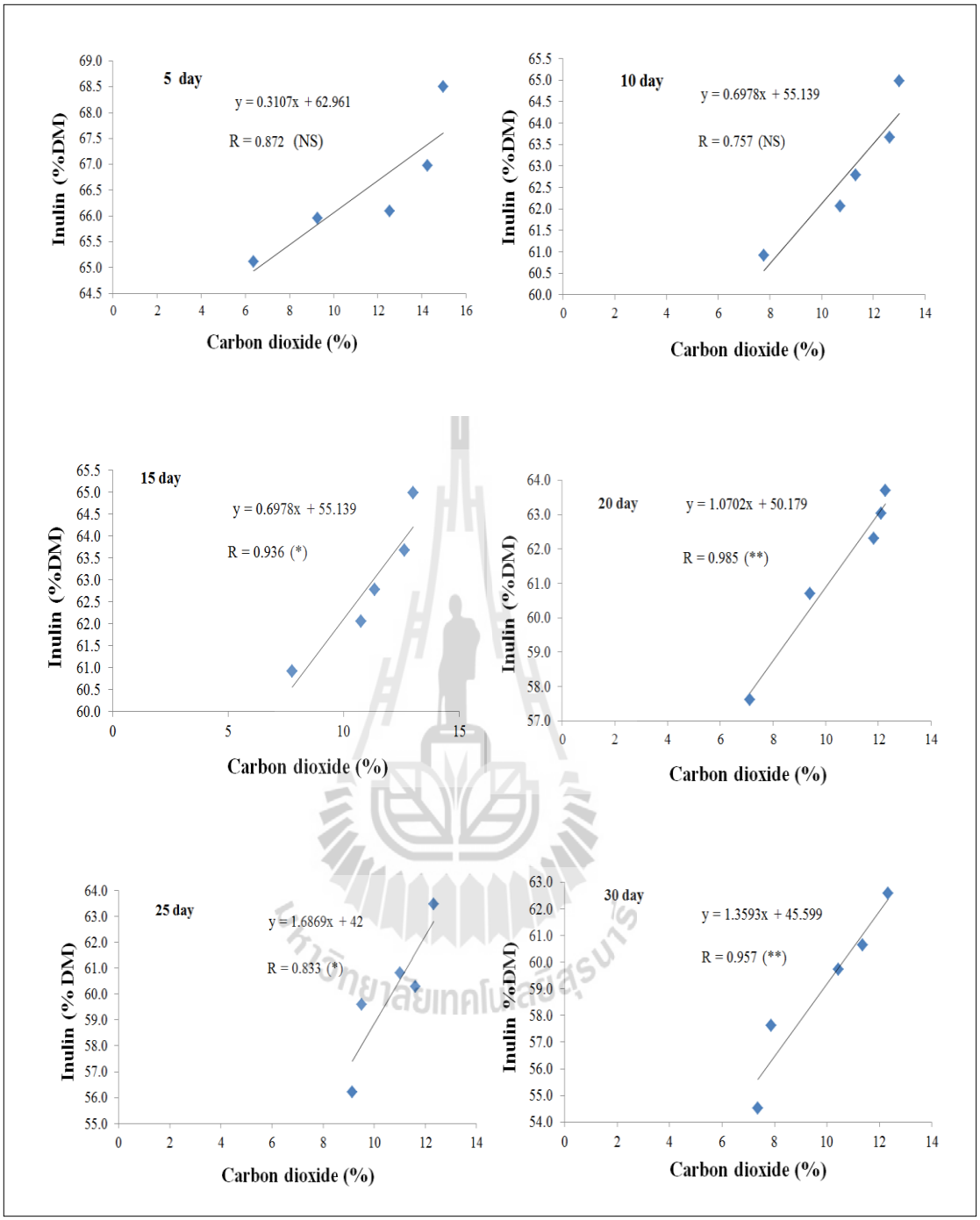

Figure 4.25 Correlations between CO_2 concentrations in the package correlated to the inulin content in the Jerusalem artichoke tubers during storage. The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

CHAPTER V

SUMMARY

Early and late harvesting periods of Jerusalem artichoke tubers resulted in different amounts of inulin. Jerusalem artichoke tubers harvested 50 days after flowering had the highest amount of inulin content, long inulin polymers, total soluble solids and dry matter content and Jerusalem artichoke tubers harvested 60 days after flowering had the highest amount of inulin content per rai, therefore, tubers harvested 50 days and 60 days after flowering seem to be the optimum harvesting periods for locally grown Jerusalem artichokes on the basis of the highest amount of inulin content, high DP inulin which appears to be the optimum harvesting periods for commercial use.

Storage at low temperature (-18 ^{o}C) resulted in a higher content of inulin in Jerusalem artichoke tubers compared to higher storage temperatures. Thus, the inulin content in Jerusalem artichokes was strongly influenced by storage temperature. During the storage of Jerusalem artichoke tubers, inulin depolymerization caused a decrease in inulin content, associated with an increase in fructose and sucrose. This effect associated with an increase of inulin hydrolase (lnH) activity and a decrease in fructan:fructan 1-fructosyl transferase (1-FFT) activity at medium and higher storage temperatures indicating higher hydrolysis and lower synthesis activities of inulin. Low temperature storage provided minimal changes in inulin content, soluble sugars, kestose, nystose, and 1-FFT activity.

Storage in MAP at 20% CO_2 resulted in an overall higher amount of inulin content in Jerusalem artichoke tubers compared to storage at 0% CO_2. Thus, inulin content in the Jerusalem artichoke tubers was strongly influenced by CO_2 concentration. During the storage of Jerusalem artichoke tubers, inulin depolymerization caused a decrease in inulin content, associated with an increase in fructose and sucrose. This effect associated with an increase in lnH activity and a decrease in 1-FFT activity during storage indicating higher hydrolysis and lower synthesis activities. High CO_2 concentrations in MAP storage minimized changes in the inulin content, fructose, sucrose, kestose, nystose, and 1-FFT activity.

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APPENDIX

Table 1A Analysis of influence of harvesting periods on inulin in Jerusalem artichoke tubers.

		Sum of Squares	df	Mean Square	F	Sig.
1-FFT	Between Groups	4985.639	4	1246.410	144.26**	.000
	Within Groups	86.398	10	8.640		
	Total	5072.037	14			
Fructose	Between Groups	220.511	4	55.128	9.43**	.002
	Within Groups	58.410	10	5.841		
	Total	278.921	14			
Kestose	Between Groups	1120.838	4	280.210	109.35**	.000
	Within Groups	25.625	10	2.563		
	Total	1146.463	14			
Nystose	Between Groups	218.352	4	54.588	26.12**	.000
	Within Groups	20.903	10	2.090		
	Total	239.254	14			
Sucrose	Between Groups	1435.718	4	358.929	279.18**	.000
	Within Groups	12.856	10	1.286		
	Total	1448.574	14			
Glucose	Between Groups	658.828	4	164.707	27.57**	.000
	Within Groups	59.741	10	5.974		
	Total	718.569	14			
Inulin	Between Groups	16026.447	4	4006.612	9.83**	.002
	Within Groups	4076.690	10	407.669		
	Total	20103.138	14			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 2A Analysis of influence of harvesting periods on tuber weight, tuber yield, dry matter, and inulin yield in Jerusalem artichoke tubers.

		Sum of Squares	df	Mean Square	F	Sig.
Tuber yield (FW)	Between Groups	273.600	4	68.400	68.40**	.000
	Within Groups	10.000	10	1.000		
	Total	283.600	14			
Tuber yield (DM)	Between Groups	132.866	4	33.217	97695.61**	.000
	Within Groups	.003	10	.000		
	Total	132.869	14			
Inulin yield/rai	Between Groups	7241471.707	4	1810367.927	952.24**	.000
	Within Groups	19011.659	10	1901.166		
	Total	7260483.366	14			
Tuber weight	Between Groups	5689540.667	4	1422385.167	6286.32**	.000
	Within Groups	2262.667	10	226.267		
	Total	5691803.333	14			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 3A Analysis of inulin profiles in Jerusalem artichoke tubers at different temperatures after 30 days storage.

		Sum of Squares	df	Mean Square	F	Sig.
DP 3-10	Between Groups	2711.624	5	542.325	545.47**	.000
	Within Groups	11.931	12	.994		
	Total	2723.554	17			
DP 11-20	Between Groups	312.518	5	62.504	82.63**	.000
	Within Groups	9.077	12	.756		
	Total	321.595	17			
DP 21-30	Between Groups	277.103	5	55.421	189.60**	.000
	Within Groups	3.508	12	.292		
	Total	280.611	17			
DP>30	Between Groups	328.192	5	65.638	761.61**	.000
	Within Groups	1.034	12	.086		
	Total	329.227	17			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 4A Analysis of glucose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Glucose 5d	Between Groups	1.335	5	.267	.638^{NS}	.675
	Within Groups	5.021	12	.418		
	Total	6.356	17			
Glucose 10d	Between Groups	32.189	5	6.438	6.529**	.004
	Within Groups	11.833	12	.986		
	Total	44.022	17			
Glucose 15d	Between Groups	4.188	5	.838	.446^{NS}	.808
	Within Groups	22.517	12	1.876		
	Total	26.704	17			
Glucose 20d	Between Groups	34.789	5	6.958	6.016**	.005
	Within Groups	13.878	12	1.157		
	Total	48.667	17			
Glucose 25d	Between Groups	5.185	5	1.037	.347^{NS}	.874
	Within Groups	35.814	12	2.984		
	Total	40.999	17			
Glucose 30d	Between Groups	9.266	5	1.853	.142^{NS}	.391
	Within Groups	19.465	12	1.622		
	Total	28.731	17			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 5A Analysis of fructose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Fructose 5d	Between Groups	3485.513	5	697.103	56.15**	.000
	Within Groups	148.979	12	12.415		
	Total	3634.492	17			
Fructose 10d	Between Groups	3657.572	5	731.514	68.83**	.000
	Within Groups	127.523	12	10.627		
	Total	3785.095	17			
Fructose 15d	Between Groups	3906.349	5	781.270	76.91**	.000
	Within Groups	121.898	12	10.158		
	Total	4028.246	17			
Fructose 20d	Between Groups	4356.012	5	871.202	15.52**	.000
	Within Groups	673.253	12	56.104		
	Total	5029.265	17			
Fructose 25d	Between Groups	6134.868	5	1226.974	125.48**	.000
	Within Groups	117.331	12	9.778		
	Total	6252.199	17			
Fructose 30d	Between Groups	8292.370	5	1658.474	35.57**	.000
	Within Groups	559.487	12	46.624		
	Total	8851.857	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 6A Analysis of sucrose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Sucrose 5d	Between Groups	1315.194	5	263.039	5.17**	.009
	Within Groups	609.990	12	50.833		
	Total	1925.184	17			
Sucrose 10d	Between Groups	1209.456	5	241.891	2.62^{NS}	.079
	Within Groups	1104.506	12	92.042		
	Total	2313.962	17			
Sucrose 15d	Between Groups	1908.906	5	381.781	3.39*	.039
	Within Groups	1350.448	12	112.537		
	Total	3259.354	17			
Sucrose 20d	Between Groups	6522.722	5	1304.544	8.68**	.001
	Within Groups	1803.235	12	150.270		
	Total	8325.957	17			
Sucrose 25d	Between Groups	6733.254	5	1346.651	13.71**	.000
	Within Groups	1178.349	12	98.196		
	Total	7911.604	17			
Sucrose 30d	Between Groups	10264.416	5	2052.883	28.13**	.000
	Within Groups	875.468	12	72.956		
	Total	11139.883	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 7A Analysis of kestose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Kestose 5d	Between Groups	746.161	5	149.232	8.43**	.001
	Within Groups	212.197	12	17.683		
	Total	958.357	17			
Kestose 10d	Between Groups	942.624	5	188.525	15.91**	.000
	Within Groups	142.134	12	11.845		
	Total	1084.758	17			
Kestose 15d	Between Groups	986.447	5	197.289	16.11**	.000
	Within Groups	146.948	12	12.246		
	Total	1133.395	17			
Kestose 20d	Between Groups	1799.742	5	359.948	83.68**	.000
	Within Groups	51.614	12	4.301		
	Total	1851.357	17			
Kestose 25d	Between Groups	1734.212	5	346.842	19.52**	.000
	Within Groups	213.187	12	17.766		
	Total	1947.399	17			
Kestose 30d	Between Groups	1978.827	5	395.765	36.14**	.000
	Within Groups	131.409	12	10.951		
	Total	2110.235	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P< 0.05$ or 0.01, respectively.

Table 8A Analysis of nystose contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Nystose 5d	Between Groups	586.108	5	117.222	23.81**	.000
	Within Groups	59.072	12	4.923		
	Total	645.180	17			
Nystose 10d	Between Groups	868.798	5	173.760	32.46**	.000
	Within Groups	64.230	12	5.353		
	Total	933.028	17			
Nystose 15d	Between Groups	553.741	5	110.748	23.98**	.000
	Within Groups	55.403	12	4.617		
	Total	609.145	17			
Nystose 20d	Between Groups	1297.876	5	259.575	31.76**	.000
	Within Groups	98.067	12	8.172		
	Total	1395.943	17			
Nystose 25d	Between Groups	1214.717	5	242.943	21.16**	.000
	Within Groups	137.750	12	11.479		
	Total	1352.467	17			
Nystose 30d	Between Groups	1518.921	5	303.784	33.22**	.000
	Within Groups	109.708	12	9.142		
	Total	1628.629	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 9A Analysis of inulin contents in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
lnulin 5d	Between Groups	5368.752	5	1073.750	.67^{NS}	.652
	Within Groups	19174.322	12	1597.860		
	Total	24543.073	17			
lnulin 10d	Between Groups	10290.530	5	2058.106	2.75^{NS}	.070
	Within Groups	8974.392	12	747.866		
	Total	19264.922	17			
lnulin 15d	Between Groups	10837.286	5	2167.457	1.355^{NS}	.307
	Within Groups	19200.678	12	1600.056		
	Total	30037.963	17			
lnulin 20d	Between Groups	17262.451	5	3452.490	2.31^{NS}	.109
	Within Groups	17936.484	12	1494.707		
	Total	35198.935	17			
lnulin 25d	Between Groups	16190.635	5	3238.127	1.88^{NS}	.172
	Within Groups	20673.553	12	1722.796		
	Total	36864.188	17			
lnulin 30d	Between Groups	23114.586	5	4622.917	7.665**	.002
	Within Groups	7237.863	12	603.155		
	Total	30352.449	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 10A Analysis of lnH in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
lnH 5 d	Between Groups	71.853	5	14.371	15.81**	.000
	Within Groups	10.902	12	.908		
	Total	82.755	17			
lnH 10d	Between Groups	435.456	5	87.091	56.93**	.000
	Within Groups	18.356	12	1.530		
	Total	453.812	17			
lnH 15d	Between Groups	607.373	5	121.475	100.91**	.000
	Within Groups	14.445	12	1.204		
	Total	621.818	17			
lnH 20d	Between Groups	500.350	5	100.070	105.71**	.000
	Within Groups	11.360	12	.947		
	Total	511.709	17			
lnH 25d	Between Groups	500.793	5	100.159	127.50**	.000
	Within Groups	9.427	12	.786		
	Total	510.219	17			
lnH 30d	Between Groups	128.922	5	25.784	138.71**	.000
	Within Groups	2.231	12	.186		
	Total	131.153	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 11A Analysis of 1-FFT in Jerusalem artichoke tubers stored at different temperatures for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
FFT 5d	Between Groups	235.482	5	47.096	12.58**	.000
	Within Groups	44.894	12	3.741		
	Total	280.376	17			
FFT 10d	Between Groups	309.589	5	61.918	16.86**	.000
	Within Groups	44.063	12	3.672		
	Total	353.652	17			
FFT 15d	Between Groups	395.941	5	79.188	11.45**	.000
	Within Groups	82.978	12	6.915		
	Total	478.920	17			
FFT 20d	Between Groups	160.106	5	32.021	7.90**	.002
	Within Groups	48.592	12	4.049		
	Total	208.698	17			
FFT 25d	Between Groups	194.819	5	38.964	31.21**	.000
	Within Groups	14.981	12	1.248		
	Total	209.800	17			
FFT 30d	Between Groups	217.043	5	43.409	30.99**	.000
	Within Groups	16.805	12	1.400		
	Total	233.848	17			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 12A Analysis of inulin profile in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
DP 3-10	Between Groups	3.349	4	.837	5.25*	.015
	Within Groups	1.592	10	.159		
	Total	4.941	14			
DP 11-20	Between Groups	2.555	4	.639	2.67^{NS}	.094
	Within Groups	2.387	10	.239		
	Total	4.942	14			
DP 21-30	Between Groups	4.682	4	1.170	12.07**	.001
	Within Groups	.969	10	.097		
	Total	5.651	14			
DP>30	Between Groups	.163	4	.041	1.51^{NS}	.271
	Within Groups	.269	10	.027		
	Total	.432	14			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 13A Analysis of inulin contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Inulin 5d	Between Groups	1999.153	4	499.788	1.50^{NS}	.273
	Within Groups	3325.358	10	332.536		
	Total	5324.512	14			
Inulin 10d	Between Groups	1553.095	4	388.274	1.074^{NS}	.419
	Within Groups	3613.545	10	361.355		
	Total	5166.640	14			
Inulin 20d	Between Groups	7061.110	4	1765.278	5.157^{NS}	.016
	Within Groups	3423.022	10	342.302		
	Total	10484.132	14			
Inulin 15d	Between Groups	2878.005	4	719.501	1.348^{NS}	.318
	Within Groups	5337.032	10	533.703		
	Total	8215.037	14			
Inulin 25d	Between Groups	8661.110	4	2165.277	1.773^{NS}	.211
	Within Groups	12213.564	10	1221.356		
	Total	20874.673	14			
Inulin 30d	Between Groups	11438.400	4	2859.600	5.410*	.014
	Within Groups	5285.936	10	528.594		
	Total	16724.336	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P< 0.05$ or 0.01, respectively.

Table 14A Analysis of fructose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Fructose 5d	Between Groups	65.848	4	16.462	2.39^{NS}	.120
	Within Groups	68.752	10	6.875		
	Total	134.599	14			
Fructose 10d	Between Groups	13.199	4	3.300	$.22^{NS}$	.919
	Within Groups	147.735	10	14.773		
	Total	160.934	14			
Fructose 15d	Between Groups	52.066	4	13.016	2.74^{NS}	.089
	Within Groups	47.484	10	4.748		
	Total	99.549	14			
Fructose 20d	Between Groups	153.257	4	38.314	1.17^{NS}	.378
	Within Groups	325.397	10	32.540		
	Total	478.655	14			
Fructose 25d	Between Groups	219.291	4	54.823	4.49*	.025
	Within Groups	122.049	10	12.205		
	Total	341.340	14			
Fructose 30d	Between Groups	287.588	4	71.897	4.58*	.023
	Within Groups	156.821	10	15.682		
	Total	444.409	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 15A Analysis of sucrose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
sucrose 5d	Between Groups	25.341	4	6.335	$.17^{NS}$	.946
	Within Groups	361.040	10	36.104		
	Total	386.382	14			
sucrose 10d	Between Groups	193.152	4	48.288	$.93^{NS}$	.480
	Within Groups	514.491	10	51.449		
	Total	707.643	14			
sucrose 15d	Between Groups	43.532	4	10.883	$.122^{NS}$	.971
	Within Groups	893.286	10	89.329		
	Total	936.817	14			
sucrose 20d	Between Groups	65.123	4	16.281	$.206^{NS}$	.929
	Within Groups	788.491	10	78.849		
	Total	853.614	14			
sucrose 25d	Between Groups	341.029	4	85.257	1.252^{NS}	.351
	Within Groups	681.088	10	68.109		
	Total	1022.117	14			
sucrose 30d	Between Groups	1511.249	4	377.812	2.318^{NS}	.128
	Within Groups	1629.678	10	162.968		
	Total	3140.927	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 16A Analysis of kestose content in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Kestose 5d	Between Groups	39.630	4	9.907	2.49 ^{NS}	.110
	Within Groups	39.653	10	3.965		
	Total	79.283	14			
Kestose 10d	Between Groups	122.683	4	30.671	13.36**	.001
	Within Groups	22.957	10	2.296		
	Total	145.641	14			
Kestose 15d	Between Groups	54.512	4	13.628	3.82*	.039
	Within Groups	35.654	10	3.565		
	Total	90.166	14			
Kestose 20d	Between Groups	65.207	4	16.302	1.86 ^{NS}	.194
	Within Groups	87.555	10	8.756		
	Total	152.762	14			
Kestose25d	Between Groups	25.832	4	6.458	.83 ^{NS}	.532
	Within Groups	77.120	10	7.712		
	Total	102.952	14			
Kestose 30d	Between Groups	142.718	4	35.680	5.61*	.012
	Within Groups	63.494	10	6.349		
	Total	206.213	14			

The notations ns, * and ** indicate non significant or significant differences at $P<0.05$or 0.01, respectively.

Table 17A Analysis of nystose contents in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
Nystose 5d	Between Groups	29.384	4	7.346	1.29 NS	.337
	Within Groups	56.923	10	5.692		
	Total	86.307	14			
Nystose 10d	Between Groups	32.852	4	8.213	.74 NS	.580
	Within Groups	109.593	10	10.959		
	Total	142.445	14			
Nystose 15d	Between Groups	29.143	4	7.286	.76 NS	.572
	Within Groups	95.256	10	9.526		
	Total	124.399	14			
Nystose 20d	Between Groups	51.657	4	12.914	1.89 NS	.188
	Within Groups	68.256	10	6.826		
	Total	119.913	14			
Nystose 25d	Between Groups	149.171	4	37.293	1.92 NS	.182
	Within Groups	193.446	10	19.345		
	Total	342.617	14			
Nystose 30d	Between Groups	42.460	4	10.615	.65 NS	.634
	Within Groups	161.039	10	16.104		
	Total	203.499	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 18A Analysis of 1-FFT in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
1-FFT 5d	Between Groups	3.556	4	.889	.98 ^{NS}	.460
	Within Groups	9.064	10	.906		
	Total	12.620	14			
1-FFT 10d	Between Groups	3.178	4	.794	2.73 ^{NS}	.090
	Within Groups	2.909	10	.291		
	Total	6.087	14			
1-FFT 15d	Between Groups	.866	4	.216	.79 ^{NS}	.556
	Within Groups	2.727	10	.273		
	Total	3.593	14			
1-FFT 20d	Between Groups	.637	4	.159	3.03 ^{NS}	.070
	Within Groups	.525	10	.053		
	Total	1.163	14			
1-FFT 25d	Between Groups	.859	4	.215	2.83 ^{NS}	.083
	Within Groups	.758	10	.076		
	Total	1.617	14			
1-FFT 30d	Between Groups	1.077	4	.269	1.94 ^{NS}	.180
	Within Groups	1.385	10	.139		
	Total	2.462	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 19A Analysis of lnH in Jerusalem artichoke tubers stored at different CO_2 concentrations for 30 days.

		Sum of Squares	df	Mean Square	F	Sig.
lnH 5d	Between Groups	28.133	4	7.033	.91 ^{NS}	.492
	Within Groups	76.838	10	7.684		
	Total	104.971	14			
lnH 10d	Between Groups	88.241	4	22.060	1.37 ^{NS}	.311
	Within Groups	160.870	10	16.087		
	Total	249.111	14			
lnH 15d	Between Groups	80.803	4	20.201	2.88 ^{NS}	.079
	Within Groups	70.029	10	7.003		
	Total	150.832	14			
lnH 20d	Between Groups	89.460	4	22.365	19.67**	.000
	Within Groups	11.366	10	1.137		
	Total	100.827	14			
lnH 25d	Between Groups	114.899	4	28.725	15.50**	.000
	Within Groups	18.523	10	1.852		
	Total	133.422	14			
lnH 30d	Between Groups	87.692	4	21.923	5.10*	.017
	Within Groups	42.940	10	4.294		
	Total	130.632	14			

The notations ns, $*$ and $**$ indicate non significant or significant differences at $P<0.05$ or 0.01, respectively.

Table 20A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 5 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	39.579	1	39.579	9.511^{NS}	.054
Residual	12.484	3	4.161		
Total	52.063	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 21A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 10 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	8.138	1	8.138	4.035^{NS}	.138
Residual	6.050	3	2.017		
Total	14.188	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 22A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 15 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	15.102	1	15.102	21.30*	.019
Residual	2.127	3	.709		
Total	17.229	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 23A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 20 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	19.436	1	19.436	100.12**	.002
Residual	.582	3	.194		
Total	20.018	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 24A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 25 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	5.824	1	5.824	10.67*	.047
Residual	1.637	3	.546		
Total	7.461	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 25A Analysis of correlations between CO_2 concentrations in the package correlated and the inulin contents in the Jerusalem artichoke tubers for 30 days storage.

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	17.293	1	17.293	33.00**	.010
Residual	1.572	3	.524		
Total	18.865	4			

The notations ns, ∗ and ∗∗ indicate non significant or significant differences at P<0.05 or 0.01, respectively.

Table 26A Modification of gases in MAP packaging of Jerusalem artichoke tubers during storage at 10 ^{o}C.

Storage (day)	Treatment		O_2 (%)	CO_2 (%)
0	1	(CO_2 0%)	21±0.00	0.1±0.00
	2	(CO_2 5%)	21±0.00	5±0.00
	3	(CO_2 10%)	21±0.00	10±0.00
	4	(CO_2 15%)	21±0.00	15±0.00
	5	(CO_2 20%)	21±0.00	20±0.00
5	1	(CO_2 0%)	1.4±0.10	6.4±0.06
	2	(CO_2 5%)	1.6±0.10	9.3±0.21
	3	(CO_2 10%)	7.8±0.21	12.5±0.25
	4	(CO_2 15%)	9.5±0.15	14.3±0.21
	5	(CO_2 20%)	9.7±0.20	14.8±0.21
10	1	(CO_2 0%)	1.4±0.12	8.8±0.20
	2	(CO_2 5%)	7.0±0.10	10.7±0.15
	3	(CO_2 10%)	7.3±0.10	12.3±0.21
	4	(CO_2 15%)	7.5±0.10	12.8±0.06
	5	(CO_2 20%)	7.9±0.15	13.3±0.17
15	1	(CO_2 0%)	1.4±0.10	7.8±0.15
	2	(CO_2 5%)	9.0±0.56	10.7±0.15
	3	(CO_2 10%)	9.4±0.10	11.3±0.15
	4	(CO_2 15%)	9.2±0.12	12.6±0.12
	5	(CO_2 20%)	9.6±0.12	13±0.10

Table 26A Modification of gases in MAP packaging of Jerusalem artichoke tubers during storage at 10 ^{o}C (Continued).

Storage (day)	Treatment		O_2 (%)	CO_2 (%)
20	1	(CO_2 0%)	1.8±0.15	7.2±0.15
	2	(CO_2 5%)	9.2±0.10	9.4±0.10
	3	(CO_2 10%)	9.9±0.21	11.9±0.15
	4	(CO_2 15%)	10.2±0.06	12.1±0.10
	5	(CO_2 20%)	10.4±0.12	12.3±0.21
25	1	(CO_2 0%)	1.9±0.38	9.1±0.21
	2	(CO_2 5%)	7.4±0.06	9.5±0.10
	3	(CO_2 10%)	7.7±0.15	11±0.26
	4	(CO_2 15%)	7.9±0.06	11.6±0.26
	5	(CO_2 20%)	8.4±0.21	12.3±0.15
30	1	(CO_2 0%)	1.6±0.38	7.4±0.15
	2	(CO_2 5%)	12.1±0.46	7.9±0.35
	3	(CO_2 10%)	12.4±0.25	10.4±0.21
	4	(CO_2 15%)	12.6±0.10	11.4±0.15
	5	(CO_2 20%)	12.8±0.15	12.3±0.15

BIOGRAPHY

Sukanya Maicaurkaew was born and brought up in Lampang province, Thailand. She attended Rajamangala University of Technology Lampang, Thailand, and received her Bachelor's degree in Food Science and Technology in 1993. She worked at International Seafood Associates (I.S.A) for 1 year. Then, she worked as a lecturer on the Food Science and Technology Program in the Science and Technology Faculty at Suratthani Rajabhat University. In 1999, she received a Master's degree in Food Technology from Prince of Songkla University, Thailand. From 2008-2013, she obtained a scholarship from Suratthani Rajabhat University and the Commission for Higher Education to pursue her Ph.D. studies at the School of Food Technology, Suranaree University of Technology, Thailand and the Whistler Center for Carbohydrate Research, Department of Food Science at Purdue University in West Lafayette, IN, USA.