INVESTIGATION OF THAI PLANTS FOR POTENTIAL SOURCES OF INULIN-TYPE FRUCTANS

SIRIPORN TANJOR

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (NUTRITION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2010**

Thesis
entitled

INVESTIGATION OF THAI PLANTS FOR POTENTIAL SOURCES OF INULIN-TYPE FRUCTANS

..….……………...……………………….
Miss Siriporn Tanjor
Candidate

..….……………...……………………….
Lect. Kunchit Judprasong, Ph.D.
Major-advisor

..….……………...……………………….
Assoc. Prof. Pongtorn Sungpuag, D.Sc.
Co-advisor

..….……………...……………………….
Assoc. Prof. Prapasri Puwastien,
Ph.D. (Emeritus)
Co-advisor

..….……………...……………………….
Prof. Banchong Mahaisavariya, M.D.
Dean
Faculty of Graduate Studies
Mahidol University

..….……………...……………………….
Prof. Surat Komindr, M.D., F.R.C.P. (T)
Program Director
Master of Science Program in nutrition
Faculty of Medicine, Ramathibodi
Hospital and Institute of Nutrition
Mahidol University

Thesis
entitled

INVESTIGATION OF THAI PLANTS FOR POTENTIAL SOURCES OF INULIN-TYPE FRUCTANS

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Nutrition)
on
February 15, 2010

..…..…………….....…………………….
Miss Siriporn Tanjor
Candidate

..…..…………….....…………………….
Assoc. Prof. Sanan Jogloy, Ph.D.
Chair

..…..…………….....…………………….
Assoc. Prof. Prapasri Puwastien,
Ph.D. (Emeritus)
Member

..…..…………….....…………………….
Lect. Kunchit Judprasong, Ph.D.
Member

..…..…………….....…………………….
Assoc. Prof. Pongtorn Sungpuag,
D.Sc.
Member

..…..…………….....…………………….
Assoc. Prof. Visith Chavasit, Ph.D.
Director
Institute of Nutrition
Mahidol University

..…..…………….....…………………….
Prof. Banchong Mahaisavariya, M.D.
Dean
Faculty of Graduate Studies
Mahidol University

..…..…………….....…………………….
Prof. Rajata Rajatanavin, M.D., F.A.C.E.
Dean
Faculty of Medicine,
Ramathibodi Hospital
Mahidol University

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and deep appreciation to my major advisor, Dr. Kunchit Judprasong, and my co-advisors, Assoc. Prof. Prapasri Puwastien and Assoc. Prof. Pongtorn Sungpuag. Without their valuable support, assistance and encouragement throughout this study, the success of this thesis would not be possible.

I would like to express my appreciation to Assoc. Prof. Sanan Jogloy from Khon Kaen University, being the chair of examination committee, for his valuable guidance and comment and also for his kindness in providing Jerusalem artichoke (Kaentawan) test samples for this study.

I would like to express my thanks to Dr. Harold C. Furr for his kindness in suggestion and editing manuscript.

I would like to express my thanks to all staffs of Food Chemistry Division especially Chanantita Chaito and Kriengkrai Vasanachitt for their helpful suggestion, technical assistance and laboratory facilities and to all my friends for their friendships and encouragement.

This thesis is partially supported by the Graduate Studies of Mahidol University Alumni Association. I wish to thank Mahidol University Alumni Association for the financial support.

Finally, I wish to express my deep appreciation to my parent and all members of the family for their love, understanding and supports which have inspired me to achieve this study.

Siriporn Tanjor

INVESTIGATION OF THAI PLANTS FOR POTENTIAL SOURCES OF INULIN-TYPE FRUCTANS

SIRIPORN TANJOR 5036837 RANU/M

M.Sc. (NUTRITION)

THESIS ADVISORY COMMITTEE : KUNCHIT JUDPRASONG, Ph.D. PONGTORN SUNGPUAG, D.Sc., PRAPASRI PUWASTIEN, Ph.D.

ABSTRACT

Inulin-type fructans are polysaccharides which have been used as potential sources of dietary fibre in many food products. Several studies found wide distribution of natural inulin and its fractions as fructo-oligosaccharide (FOS) in a variety of plants such as starchy roots, fruits, and vegetables. However, there has been a lack of information about foods consumed in Thailand. Therefore, the objective of this study was to determine inulin-type fructans, FOS (1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF_4)) and sugars (glucose, fructose and sucrose) in potential food sources. For preliminary study, forty-seven species of Thai plant foods, distributing in five food groups, were selected and purchased from one representative market. Inulin-type fructans, FOS and total sugars were extracted following the method 997.08 of AOAC and these components were determined by gas chromatography. Potential food sources of inulin-type fructans and FOS were identified. Two more sets of identified samples were randomly purchased from two other representative markets and the same analyses were conducted. High levels of inulin-type fructans were found in great headed garlic, Chinese garlic, common garlic and Jerusalem artichoke (Kaentawan) (29.16$\pm$5.62, 24.29$\pm$1.94, 22.44$\pm$2.86 and 19.36$\pm$1.04 g/100g fresh weight (FW), respectively), and medium levels were found in shallot and red onion (8.86$\pm$0.75 and 3.56$\pm$0.95 g/100 g FW, respectively). High levels of FOS were found in Jerusalem artichoke, shallot and red onion (5.18$\pm$0.04, 4.98$\pm$0.51, 3.09$\pm$0.54 g/100 g FW, respectively). The additional information about inulin-type fructans and FOS in Thai plants would be beneficial to consumers, academics and health professional.

KEY WORDS: INULIN-TYPE FRUCTANS/ FRUCTO-OLIGOSACCHARIDES/ THAI FOODS

72 pages

การศึกษาพืชของไทยที่เป็นแหล่งของฟรุกแตนซึ่งมีอินนูลินเป็นองค์ประกอบที่สำคัญ
INVESTIGATION OF THAI PLANTS FOR POTENTIAL SOURCES OF INULIN-TYPE FRUCTANS

ศิริพร ตันจอ 5036837 RANU/M

วท.ม. (โภชนศาสตร์)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: ครรชิต จุดประสงค์, Ph.D., พงศธร สังข์เผือก, D.Sc., ประภาศรี ภูวเสถียร, Ph.D.

บทคัดย่อ

ฟรุกแตนซึ่งมีอินนูลินเป็นองค์ประกอบที่สำคัญ เป็นโพลีแซคคาไรค์ชนิดหนึ่งที่มีการนำมาใช้เป็นส่วนประกอบหนึ่งในอาหารหลายผลิตภัณฑ์ มีหลายการศึกษาที่พบฟรุกแตนและฟรุคโตโอลิโกแซคคาไรค์ในพืชประเภทพืชหัวกลุ่มที่เป็นแหล่งสะสมของแป้ง ผลไม้ และผักชนิดต่างๆ อย่างไรก็ตามยังไม่มีข้อมูลสารเหล่านี้ในอาหารที่รับประทานได้ในประเทศไทย ดังนั้นวัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อศึกษาและวิเคราะห์หาปริมาณฟรุกแตน, ฟรุคโตโอลิโกแซคคาไรค์ชนิดต่างๆ และน้ำตาล ในพืชที่น่าจะเป็นแหล่งของสารเหล่านี้ ได้ทำการศึกษาเบื้องต้นด้วยการคัดเลือกพืชที่น่าจะเป็นแหล่งอาหารของฟรุกแตน และฟรุคโตโอลิโกแซคคาไรค์จำนวน 47 ชนิด ใน 5 กลุ่มอาหาร จากตลาดแห่งหนึ่ง วิเคราะห์ด้วยวิธีมาตรฐานสากล (AOAC Method 997.08) และตรวจวัดปริมาณสารเหล่านี้ด้วยเครื่องแก๊สโครมาโตกราฟี ผลจากการศึกษาเบื้องต้นนำมาพิจารณาคัดเลือกพืชที่มีปริมาณฟรุกแตนค่อนข้างสูงเพื่อทำการสุ่มซื้อจากตลาดอีก 2 แห่งโดยดำเนินการวิเคราะห์แบบเดียวกัน ผลการศึกษาพบตัวอย่างที่มีฟรุกแตนสูง ได้แก่ กระเทียมโทนหัวใหญ่, กระเทียมจีน, กระเทียมไทย และแก่นตะวัน (29.16$\pm$5.62, 24.29$\pm$1.94, 22.44$\pm$2.86 และ 19.36$\pm$1.04 กรัมต่อ100 กรัมตัวอย่างสด ตามลำดับ) และพบตัวอย่างที่มีปริมาณฟรุกแตนปานกลางคือ หอมแดง และหอมแขก (8.86$\pm$0.75 และ 3.56$\pm$0.95 กรัมต่อ100 กรัมตัวอย่างสด ตามลำดับ) ส่วนฟรุคโตโอลิโกแซคคาไรค์พบปริมาณสูงในแก่นตะวัน, หอมแดง และหอมแขก (5.18$\pm$0.04, 4.98$\pm$0.51 และ 3.09$\pm$0.54 กรัมต่อ100 กรัมตัวอย่างสด ตามลำดับ) ผลจากการศึกษาครั้งนี้ทำให้ได้ข้อมูลของฟรุกแตน และฟรุคโตโอลิโกแซคคาไรค์ในพืชของไทยซึ่งข้อมูลเหล่านี้น่าจะเป็นประโยชน์กับผู้บริโภค งานด้านการเรียนการสอน และบุคคลที่ทำงานเกี่ยวกับสุขภาพ

72 หน้า

CONTENTS

CONTENTS (cont.)

CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
°C	degree celsius
d	day
DP	degree of polymerization
FID	flame ionization detector
FOS	fructo-oligosaccharide
FW	fresh weigh
GC	gas chromatography
g	gram
GF_2	1-kestose (1-kestotriose)
GF_3	nystose (1,1-kestotetraose)
GF_4	1^F-β fructofuranosylnystose (1,1,1-kestopentaose)
HPLC	high performance liquid chromatography
hr.	hour
HCl	hydrochloric acid
INMU	Institute of Nutrition, Mahidol University
Kcal	kilocalorie
kg	kilogram
KOH	potassium hydroxide
µg	microgram
mg	milligram
ml	milliliter
min.	minute
N	Normality
ND	not detected
rpm	round per minute

LIST OF ABBREVIATIONS (cont.)

SCFA	short chain fatty acids
SD	standard deviation
TSIM	tri- methysilylimidazole

CHAPTER I
INTRODUCTION

Cardiovascular disease, obesity, diabetes and osteoporosis are public health problems which are becoming more prevalent in Thailand. Increasing life expectancy of the Thai people together with changes in life style and eating habits that occurred as the country developed into a more industrialized nation may have contributed to the apparent increase in the incidence of these problems. The health consequences increase risk of premature mortality, to serious chronic conditions that reduce the overall quality of life of Thai people [1]. Dietary fiber has emerged as one of the dietary factors in the prevention and treatment of chronic diseases. Because of the structure of dietary fiber, it is not digested in the human stomach and small intestine but it is fermented by the bacteria in the large intestine. Products of this fermentation can increase solubility and absorption of minerals (especially calcium). Consumption of dietary fiber could help to normalize blood glucose and insulin levels, reduce blood cholesterol levels and improve bone mineral density which appear to be significantly lower risk factors for developing coronary heart disease, diabetes, obesity and osteoporosis, respectively [2-4].

Inulin and fructo-oligosaccharide (FOS) are some of a group of carbohydrate substances, namely polysaccharides which are used for several nutritional reasons: 1) as a soluble dietary fiber with low caloric value and health related benefits [5]. These substances have been claimed to improve some physiological functions in humans such as relieving constipation [6], decreasing the risk of osteoporosis (by increasing mineral absorption, especially of calcium) [7], and atherosclerosis (by lowering the synthesis of triglycerides and reducing plasma cholesterol concentrations) [8]; 2) as prebiotics which stimulate the immune system of the body by stimulating beneficial gut microflora unlike most dietary fiber [9]. Eventually, several nutritional properties of these substances provide healthful benefits and prevent the risk of many diseases.

Inulin and FOS are found to be widely distributed in varieties of plants as plant storage carbohydrates, i.e., starchy roots, fruits, and vegetables [10, 11]. The interest in these substances is increasing especially for the use of commercial inulin in various food industries. They are used as dietary fiber supplements in functional beverages or as food ingredients in several food products e.g. dairy products, infant and follow-on formula products and meal replacers [12, 13].

Although inulin and FOS are distributed in a variety of plant foods [10, 11], information on these component in common foods in Thailand is not available. The objective of this study was to determine inulin-type fructans, fructo-oligosaccharides (1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF_4)) and sugars (fructose, glucose and sucrose) in potential food sources. The data will be used to estimate the intake of inulin and FOS in selected plants for daily consumption of Thai people. The potential food sources for inulin and FOS should be promoted for consumption and developed as sources of dietary fiber fortification in commercial products in Thailand.

CHAPTER II
OBJECTIVES

The objectives of this study:

General objective:

To determine inulin-type fructans, fructo-oligosaccharides (1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1F-β-fructofuranosylnystose (1,1,1-kestopentaose; GF_4)) and sum of sugars (fructose, glucose and sucrose) in potential food sources.

Specific objectives:

1. To analyse inulin-type fructans and fructo-oligosaccharide components in selected Thai plant foods.
2. To analyse sugar contents (fructose, glucose and sucrose) in selected Thai plant foods.
3. To identify potential sources of inulin-type fructans and fructo-oligosaccharide in Thai plant foods.

CHAPTER III
LITERATURE REVIEW

3.1 Definition of fructan, inulin and fructo-oligosaccharide

Fructans are one of a group of carbohydrate substances, namely polysaccharides in which fructososyl-fructose links constitute the majority of the glycosidic bonds. They are linear or branched fructose polymers, which are either β (2-1) linked inulin (mainly of plant origin) or β (2-6) linked levans (major products from some fungi and many bacteria) [14, 15]. Types of carbohydrates are shown in **Table 1.**

Inulin, one of the types of fructans, is a linked fructose polymer with β (2-1) glycosidic bonds. It contains a mixture of fructose polymers with a chain length from 2 to 60 units (degree of polymerization; DP) usually with a terminal unit of glucose. In general, formation of inulin has two classes; fructosyl-fructose linkage with a glucose unit (GF_n-type) and only fructosyl-fructose linkage (F_n-type) where *G* is a glucose moiety, *F* is a fructose moiety and *n* is the number of fructose molecules.

Oligofructose is a result of partial enzymatic hydrolysis as a short chain of inulin which has a chain length from 2 to 10 units (DP <10) [14-17].

Table 1. Types of carbohydrates

Class (DP*)	**Group**	**Components**
Sugars (1-2)	Monosaccharides	Glucose, galactose, fructose
	Disaccharides	Sucrose, lactose, maltose
Oligosaccharides	Malto-oligosaccharides	Maltodextrins
(3-9)	Other oligosaccharides	Fructo-oligosaccharide (FOS),
		Galacto-oligosaccharide (GOS),
		Mannan-oligosaccharides (MOS)
Polysaccharides	Starch	Amylose, amylopectin
(>9)	Non-starch	Fructans;
		Inulin (β 2-1), levans (β 2-6)
		Mannan, galactan, chitin

DP* = Degree of polymerization

Adapted from; FAO/WHO 1998 [18] and Wikipedia, the free encyclopedia.mht [19].

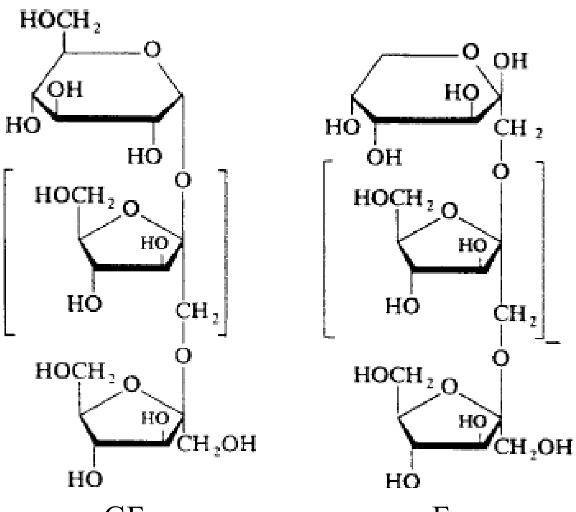

Figure 1. Chemical structures of inulin (GF$_n$ and F$_n$)

G = glucosyl; *F*= frucosyl; *n* is the number of fructose molecules [20]

Structurally, different oligosaccharides have been referred to as oligofructose such as fructo-oligosaccharides (FOS). It contains a mixture of oligosaccharides consisting of glucose linked to two, three, or four fructose units (DP 3-5) as well as a 1-kestose (1-kestotriose; GF_2), nystose (1,1-kestotetraose; GF_3), and 1^F-β fructofuranosylnystose (1,1,1-kestopentaose; GF_4) (**Figure 2**) [21].

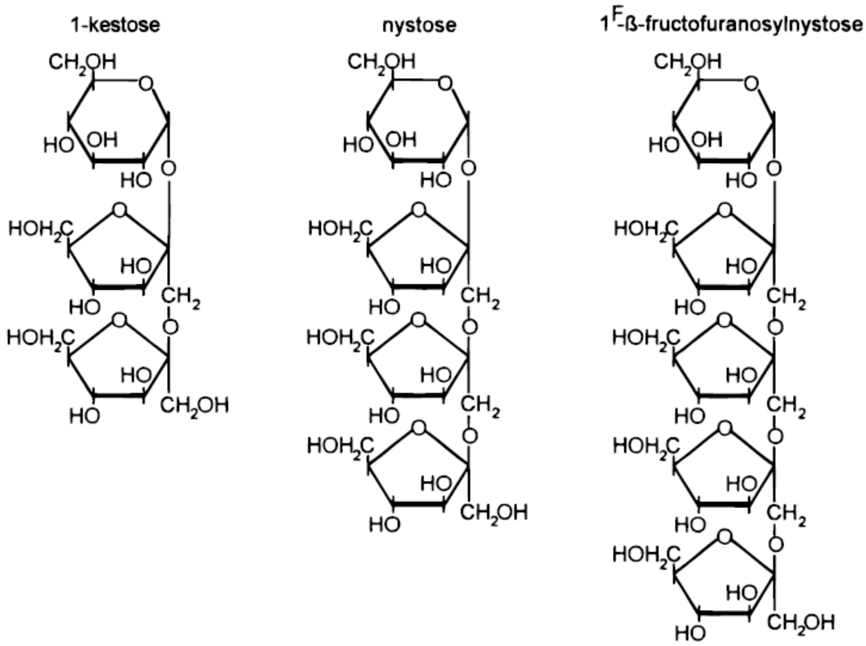

Figure 2. Molecular structures of the fructo-oligosaccharide (FOS) [21]

3.2 Food sources of inulin-type fructans

Naturally occurring inulin and FOS are found widely distributed in varieties of plants as plant storage carbohydrates, i.e., starchy roots, fruits, and vegetables such as chicory root, Jerusalem artichoke, banana, onion, leek, garlic, wheat, barley and asparagus [10, 11] as shown in **Table 2-4**.

Jerusalem artichoke is one of the inulin sources for industrial production, inulin content ranging 16-20% [10]. Interest in Jerusalem artichokes has increased because of its potential as a source of inulin. The plant is native to Canada and it is well known in Canada, the United States, and European countries under the name "sunchoke". However, the levels of inulin and FOS in Jerusalem artichoke might vary due to plant varieties, harvest time and various conditions of cultivation as well as the moisture content in each species [22, 23].

In Thailand, Jerusalem artichoke is called by the Thai name as "Kaen-ta-wan", it can be adapted to grow and resist in hot weather fields at Khon Kaen University Agronomy Farm [24]. It is a herbaceous plant, its tuber is eaten which is like ginger or galingale. Hence, it should be a potential source of inulin and fructo-oligosaccharides in terms of production in Thailand. The tuber of Kaentawan can be consumed fresh in salad or cooked in the same way as potato such as boiled or fried Kaentawan. Contents of inulin-type fructans and individual sugars of Jerusalem artichoke or Kaentawan (*Helianthus tuberosus* L.) are shown in **Table 5.**

Table 2. Inulin and oligofructose content of some foods

Type of plants	Inulin (g/100g)	Oligofructose (g/100g)
onion	1.1-7.5	1.1-7.5
Jerusalem artichoke	16.0-20.0	12.0-15.0
chicory	35.7-47.6	19.6-26.2
leek	3.0-10.0	2.4-8.0
garlic	9.0-16.0	3.6-6.4
asparagus	2.0-3.0	2.0-3.0
banana	0.3-0.7	0.3-0.7
wheat	1.0-4.0	1.0-4.0
rye, baked	0.5-0.9	0.5-0.9
barley	0.5-1.0	0.5-1.0
dandelion	12.0-15.0	9.6-12.0

Source: Van Loo et al., 1995 [10]

Table 3. Fructo-oligosaccharide composition of fruits

Foods	mg/g of DM			
	GF_2 [a]	GF_3 [b]	GF_4 [c]	Total [d]
banana	5.9	0.1	0.0	6.0
banana, green	3.1	0.0	0.0	3.1
banana, ripe	8.6	0.0	2.3	10.9
peach	3.5	0.0	0.0	3.5
watermelon	2.8	0.0	0.1	3.0

[a] 1-kestose. [b] Nystose. [c] 1F-β- Fructofuranosylnystose. [d] Total fructo-oligosaccharide.

Source: Campbell et al., 1997 [11]

Table 4. Fructo-oligosaccharide composition of vegetables

Foods	**mg/g of DM**			
	GF_2 [a]	**GF_3 [b]**	**GF_4 [c]**	**Total [d]**
Artichoke, globe	13.4	5.5	2.8	21.8
asparagus	0.3	0.0	0.0	0.3
carrot, Bunny	2.2	0.0	0.0	2.2
carrot, Dole	1.4	0.0	0.0	1.4
chicory root, raw	9.1	6.1	5.9	21.0
Chinese chive	0.4	0.3	0.4	1.1
eggplant	0.2	0.4	0.2	0.6
garlic	8.7	1.2	0.4	10.3
ginger root	0.0	0.0	0.0	0.0
Jerusalem artichoke	93.9	94.3	98.1	286.2
leek	3.4	0.6	0.7	4.8
lettuce	4.9	1.9	1.1	7.9
onion, red	11.7	2.1	0.9	14.7
onion, welch	5.8	3.9	3.6	13.4
onion, white	17.1	8.8	6.1	32.0
onion, yellow	15.5	6.7	4.2	26.4
peas	0.2	0.2	0.4	0.7
potato, sweet	0.8	0.0	0.0	0.8
radish, red	0.0	0.0	3.0	3.0
shallot	28.2	14.2	10.6	52.9
taro root	0.1	0.0	0.0	0.1
tomato	0.0	0.0	0.0	0.0
yam	0.9	0.0	0.0	0.9

[a] 1-kestose. [b] Nystose. [c] 1F-β- Fructofuranosylnystose. [d] Total fructo-oligosaccharide.

Source: Campbell et al., 1997 [11]

Table 5. Sugar and fructan content of Kaentawan (*Helianthus tuberosus* L.)

Type of sugars	**Content (g/100g)**
Fructose	3.8
Glucose	5.1
Sucrose	14.5
DP3	10.6
DP4	10.2
DP5	7.5
DP6	7.0
DP7	6.1
DP8	5.3
DP8>	29.9

Source: Khon Kaen Agriculture Journal (2006) [24]

3.3 Physiological effects and nutritional properties

3.3.1 Inulin-type fructans as dietary fiber

The basic attributes of a dietary fiber are resistance to hydrolysis by human alimentary enzymes, resistance to absorption in the small intestine, hydrolysis and fermentation (partial or total) by the bacteria in the large bowel [6].

Inulin and oligofructose are metabolised in the same way as dietary fibers that cannot be digested by the enzymes of the human small intestine because it has no enzyme to hydrolyse the glycosidic linkages β (2→1). Thus, they have been classified as ‘non-digestible carbohydrates’, or soluble dietary fibers [25-29], and they have shown various beneficial effects as follows:

a) Low energy value and weight control

Being non-digestible carbohydrates, inulin and oligofructose are not hydrolysed in the stomach or small intestine so their caloric values are significantly lower than these of digestible carbohydrates, but they are totally fermented by colonic microflora. The short-chain fatty acids and lactate produced by fermentation contribute only 1.5 kcal per gram of inulin or oligofructose [5, 25]. For food technology, inulin is used as a fat replacer in dairy products (such as milk, drinking yoghurt) leading to lower energy consumption. Therefore, they are suitable for weight control diets.

b) Low glycemic index and improved glucose regulation

Since inulin and oligofructose are non-digestible carbohydrates which are not broken down into simple sugars by the normal digestion system, so they do not raise blood sugar (very low glycemic index response) [30]. Evidently appetite regulation by glycemic index could also have an effect on heart health and obesity [31]. Moreover, the glycemic index of the food also has an effect on the incidence of non-insulin dependent diabetes. In diabetic subjects, taking 8 g of FOS per day for 14 days led to a decrease in fasting blood glucose [32]. The better blood sugar levels can be controlled, the fewer the long-term complications of diabetes that may occur. Therefore, supplementary inulin and oligofructose are interested in the treatment and preventing of the risk of obesity and diabetic.

c) Relieving constipation

Inulin and oligofructose as dietary fibers have positive effects on basic physiological functions of the colon, i.e., stool production and fecal excretion. A meta-analysis of the published data revealed that consuming inulin-type fructans significantly increased fecal biomass and stool frequency in subjects receiving 15-40 g of inulin per day for 2 weeks [9, 33-35]. In addition, they tended to increase faecal bulk with 1.5 to 2 g per g inulin intake. Therefore, inulin and oligofructose should have a significant role in relieving constipation [6, 36].

d) Improvement of the bioavailability of calcium

The effect of inulin and oligofructose on mineral absorption is mainly due to their dietary fiber characteristics. The acidification of the lumen of the colon, by this fermentation by the colonic micro flora of inulin and oligofructose, through the production of short chain fatty acids (SCFA) (essentially acetate, butyrate and propionate and other organic acids, e.g. lactate) contributes to a lower luminal pH in the large intestine. Therefore, they increase solubility and absorption of calcium, so that the bioavailability of calcium is increased [25]. Passive diffusion of Ca in the intestinal part of the small intestine and the beginning of the large intestine is thus improved [37, 38]. Emergent evidence shows that inulin and oligofructose can improve Ca absorption in children and teenagers in their growth years. The Ca absorption was increased significantly in subjects supplementing with 15 - 40 g/day of inulin or oligofructose [7, 39]. The minimum effective daily dose to achieve a significant effect has been confirmed at the ingestion of 8 g per day of oligofructose-enriched inulin (a combination of short and long-chain inulin). It showed a significant increase in calcium absorption and enhanced both whole-body bone mineral content and whole-body bone mineral density after one year of supplementation in adolescents [40].

For post-menopausal women, deficiency of estrogen at menopause decreases intestinal Ca absorption which contributes to a negative Ca balance and bone loss. Holloway et al. [41] studied mineral absorption in fifteen post-menopausal women (72.2 years). These results suggested that 6 weeks with 5 g of SYN1 treatment (mixture of oligofructose with long-chain inulin) can improve mineral absorption and impact markers of bone turnover in post-menopausal women. Long-term beneficial effects on bone of inulin and oligofructose rich diet have been indicated by increasing mineral absorption, preventing bone loss and decreasing risk factors responsible for osteoporosis. Therefore, the beneficial effects of inulin and oligofructose target not only the mineral absorption phase but also other aspects of bone health, especially bone mineralisation or bone density, bone accretion and resorption.

e) Improvement of lipids and cholesterol metabolism

As soluble dietary fiber, inulin and oligofructose are completely fermented by the colonic microflora and produce gases. Short chain fatty acid (SCFA), essentially acetate and propionate, are produced to regulate normalisation of blood lipids. Inulin and oligofructose (daily intake 8-20 g of inulin) have a beneficial effect on human lipid metabolism by reduction of serum cholesterol concentrations and triglycerides [30, 32, 33, 42, 43]. A clinical trial by Brighenti et al. [33] found a marked reduction in the plasma triglyceride levels and a moderate decrease in plasma cholesterol in twelve men consuming 9 g of inulin per day. In addition, the study of Letexier et al. [44] demonstrated reduced hepatic lipogenesis, plasma triglycerol concentrations and cholesterol synthesis in eight control subjects, receiving 10 g/day of inulin for 3 week. From these results, the use of inulin and oligofructose as soluble dietary fiber may have an effect on reducing risk factors for help in the treatment and prevention of many diseases such as coronary heart disease and atherosclerosis.

f) Reduction of risk of colon carcinogenesis

Several studies in experimental animal models revealed that inulin and oligofructose have anti-cancer properties. In human cells, derived fermentation products of inulin can inhibit cell growth, modulate differentiation and reduce metastasis activities, which reduce the risks of colon cancer [45, 46]. In a human intervention study, volunteer patients consumed a synbiotic, composed of a prebiotic (oligofructose-enriched inulin) and two probiotics (*Lactobacillus GG* and *Bifidobacterium Bb12*) added to the diet, for 3 months which reduced risk factors for colon cancer [47].

3.3.2 Inulin-type fructans as prebiotics

The classification of a food or a food ingredient as prebiotic requires the scientific demonstration of resistance to gastric acidity, hydrolysis by human enzymes, and gastrointestinal absorption, fermentation by intestinal microflora, and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being [20].

Inulin and oligofructose cannot be digested in the small intestines and so are passed down into the colon. However, the properties of inulin and oligofructose are unlike most other dietary fibers, they are fermented and bacterial production goes to work by selectively stimulating the growth of specific microflora in the colon. The fermentation of inulin-type fructans has been shown to have beneficial health effects on the human gut microbiota both *in vitro* and *in vivo* by selectively promoting the growth of bifidobacteria and suppressing pathogenic organism [9, 20, 48-50]. To maintain a well balanced bacteria flora, there must be more beneficial or “good bacteria” than pathogenic bacteria, therefore it’s essential to promote the growth of good bacteria. These confer benefits upon host well-being and health as “prebiotics”.

For a given chain length, there is no difference in fermentation rate between GF(n) and F(n)- types of fructan. However, the higher solubility of FOS compared to polymeric inulin (DP > 10) makes it more easily available and therefore more rapidly fermentable by the microbiota in the colon [29, 51]. Numerous human studies have demonstrated this effect starting from 5 g per day [52], and including an intervention for over 2 months [53]. For example, doses of 15 g/day inulin or oligofructose were efficient in stimulating the growth of these bacteria, classified in the balance of microbiota in the large intestine as potentially beneficial for health; especially oligofructose both stimulates the growth of bifidobacteria and reduces the count of bacteroids, fusobacteria and clostridia [20]. The study of Langlands et al. [54] found that fourteen subjects who supplemented their diet for 2 weeks with a mix of 7.5 g of oligofructose and 7.5 g inulin, showed increases in both bifidobacteria and lactobacilli counts in the mucosa. In addition, inulin and oligofructose not only increased the quantity of microflora, but also improved the immunity in the human body, especially in persons having symptoms of allergy [55]. In a recent clinical trial, thirty-five infants attending day-care centers (age 7-12 mounts) that received oligofructose (2 g/day) for 42 days showed increased resistance to infections and had increased well-being [56].

3.4 Typical inulin and oligofructose intake and side effects

Estimates of daily inulin consumption from various natural foods range from 1 to 4 g for Americans and up to 11 g for Europeans [10]. According to the U.S. Department of Agricultural 1994-1996 Continuing Survey of Food Intakes by Individuals, American diets provide 2.6 g of inulin and 2.5 g of FOS per day [57]. The most commonly consumed foods containing FOS included globe artichoke, banana, garlic, onion, and several cereal grains. However, the recommended daily intake (RDI) of inulin- type fructans and FOS has not yet been established. In commercial products, fortification of inulin or FOS is usually in the range of 3 to 6 g per portion, in extreme cases up to 10 g [58]. Inulin or oligofructose is added to allow a specific nutritional claim such as that regarding the healthy bacterial flora, at typical levels 3–8 g per portion [58]. Inulin is added at about 1.5 g per serving in infant milk powder [59]. For diabetic and hyperlipidemic (cholesterol and triglyceride) patients, the usual recommendation ranges from 8 to 20 grams per day.

Human tolerance of inulin and FOS is primarily dictated by chain length and dosage. Abdominal symptoms, primarily gas and some abdominal discomfort, increases with increasing dose and decreasing chain length [60]. In an experiment to study the increase in tolerance of FOS (DP 3-7), the maximum daily dose that did not cause gastrointestinal symptoms was 27 to 31 g for men and 33 to 37 g for women [61]. Takahashi et al. [62] found that the maximum dose which did not cause diarrhea was approximately 21 to 24 g per day. Coussement [58] recommended a formulation doses, based on tests with volunteers and his experience in the food industry, at a level of 5–8 g per portion for oligofructose and 10 g for inulin.

3.5 Inulin-type fructans and importance to the food industry

Inulin and oligofructose are used as dietary fiber supplements [13, 65]. Because they are not digested in the upper gastrointestinal tract, products with inulin and oligofructose added have a reduced caloric value. They not only have more nutritional properties but also can improve the textural properties of food, and substitute part of the sugar or fat content in a wide range of products like pastry, dairy products chocolate and ice cream [12]. In Thailand, inulin and oligofructose are added as ingredients in many food products present on supermarket shelves such as dairy products (e.g. drinking yoghurt), infant and follow-on formula products, and diet foods. They are used as dietary supplements with nutritional properties for relieving constipation and stimulating the immune system.

Commercial inulin and oligofructose are obtained and purified from chicory roots. Fructo-oligosaccharides (FOS) are synthesized from sucrose. For example, Beneo^{TM} inulin and oligofructose are extracted from chicory roots using hot water. Chicory is a plant whose tuberous roots store inulin. Oligofructose is obtained through partial enzymatic hydrolysis of inulin. Generally, the product with DP from 2 to 60+ is labeled as inulin (Raftiline), whereas oligofructose is defined as DP < 10 (Raftilose) [12].

Inulin can stabilise water into a creamy structure with the same mouthfeel as fat. This enables product developers to replace part of the fat content in developing low-fat food products. Typically, 1 g of fat is replaced by a 0.25 g of inulin.

Consequently, fat replacement in most foods will lead to inulin concentrations of ~2 to 6 g per portion [58]. Oligofructose is a natural sugar replacement (sweetening power). Its energy value is theoretically lower than that of sucrose and it has a moderately sweet taste, less than 30% the sweetness potential of sucrose. It is used as a sugar substitute mainly in dairy products and bakery products. At levels of 2 to 6 g per portion which are used frequently without intestinal discomfort.

In application of inulin in other studies, it has functional properties such as the ability to act as a fat replacer without adversely affecting food flavor, for example, for modified ice cream [64] and wheat flour mix for bread [65]. It is possible to use inulin as a fat replacer in modified products, starting at 5 % by weight.

3.6 Analysis of inulin-type fructans

The increasing use of inulin and oligofructose as ingredients in food products has importance for the development of several methods for the determination of inulin and oligofructose in plant and food products [66]. The standard analytical method for total dietary fiber analysis, AOAC Official Method 985.29 [67], is not suitable for measuring inulin or oligofructose because these substances are partially precipitated in the alcohol treatment step. As a lower molecular mass dietary fiber, it is not completely precipitated by 95% ethanol, so it cannot be assessed by the standard AOAC method used for dietary fiber analysis.

In future, inulin and oligofructose as novel dietary fibers might have significant consequences for nutrition labeling systems [58] as shown in **Figure 3**.

AOAC International has adopted the fructan method that allows specifically the accurate quantitative determination of inulin and oligofructose in foods. The general principle of the method, in brief, includes extraction of inulin with hot water, followed by hydrolysis with inulinase enzyme, and determination of the released fructose and glucose. The difference between the content of each sugar with and without enzyme hydrolysis is the amount of fructan (mostly inulin) in the food sample as Method 997.08 [67].

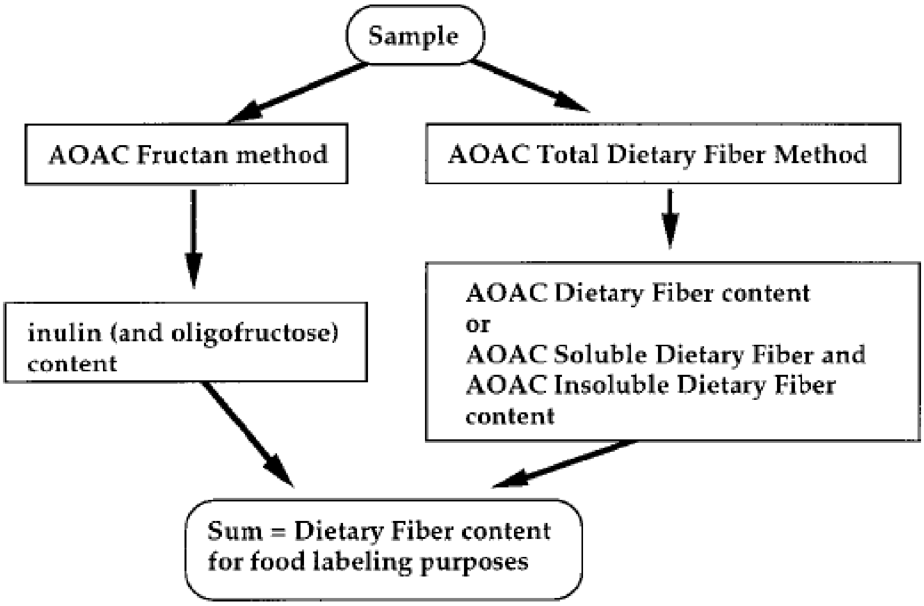

Figure 3. Schematic presentation of AOAC methods for dietary fiber determination including inulin and oligofructose.

Several methods have been published for the determination of inulin and FOS using ion-exchange chromatography [68], enzymatic/spectrophotometry [69], high-performance liquid chromatography [70], and gas chromatography [71]. However, gas chromatography (GC) may be more complex than LC and HPAEC because of the comprehensive sample pretreatment to derivatise the sugar. A high-temperature capillary gas chromatographic method can determine not only simple sugars but also individual oligomers (with DP <10) in foods and food products.

Gas chromatography shows accurate results for determination of inulin and FOS in foods and can quantify the individual oligomers up to DP10 as well as showing less interference from other sugars. Sample preparation involves sugar extraction and then derivatisation by oxymation and silylation of sugars. The oximetrimethylsilyl derivatives are extracted using isooctane and analysed on an apolar capillary aluminum-clad column, with temperature programming up to 440 ºC and detection by flame ionization. This method shows accurate results, with excellent recovery of spiked samples and repeatability.

CHAPTER IV
MATERIALS AND METHODS

This study was divided into 2 parts: preliminary study of inulin-type fructans, fructo-oligosaccharides (FOS) and sugars in various Thai foods and study of the components in potential food sources.

4.1 Food sampling

For preliminary study with the objective to identify potential food sources of inulin, forty-seven varieties of plants foods, distributed in five food groups, were selected as test samples. They were 12 kinds of starchy roots and tubers, 13 kinds of fruity vegetables, 7 kinds of fruits, 11 kinds of spices, and 4 kinds of seeds, as shown in **Table 6**. The selected foods (except Jerusalem artichoke) were randomly purchased from three retailed shops at Salaya market (as a representative market selling fresh foods from the southern part of Thailand). Two more sets of potential food sources, identified in from the preliminary study were sampled again from two more markets in Bangkok, Thailand (Mahanak and Saphanmai markets, as a representative markets selling fresh foods from the central part and the northern part of Thailand, respectively). A common variety of Jerusalem artichoke or Kaentawan was randomly colleted from Khon Kaen University Agronomy Farm, Thailand, as shown in **APPENDIX A**. Two more varieties of Jerusalem artichoke were collected again from the same places.

Table 6. List of studied foods (common name, Thai name and scientific name)

Common name	Thai name	Scientific name
Starchy roots and Tubers		
Cassava	Man sam-pa-lang (มันสำปะหลัง)	*Manihot esculenta* Crantz
Jerusalem artichoke	Kaen-ta-wan (แก่นตะวัน)	*Helianthus tuberosus* L.
Noh kala shoot	Nor-ka-la (หน่อกะลา)	*Alpinia nigra* B.L. Burtt
Potato	Man-fa-rang (มันฝรั่ง)	*Solanum tuberosum* L.
Sweet potato	Man-thet (มันเทศ)	*Ipomoea batatas* (L.) Lam
Sweet potato, red	Man-tor-phueak (มันต่อเผือก)	*Ipomoea batatas* (L.) Lam
Sweet potato, white	Man-leung (มันเหลือง)	*Ipomoea batatas* (L.) Lam
Sweet potato, yellow	Man-kai (มันไข่)	*Ipomoea batatas* (L.) Lam
Taro, hom variety	Phueak-hom (เผือกหอม)	*Colocasia esculenta* (L.) Schott
Taro, small size	Phueak (เผือก, เล็ก)	*Colocasia esculenta* (L.) Schott
Yam bean	Man-kaeo (มันแกว)	*Pachyrhizus erosus* (L.) Urb.
Yam, dried	Kloi (กลอย)	*Dioscorea hispida* Dennst.
Vegetables		
Asparagus	No-mai-fa-rang (หน่อไม้ฝรั่ง)	*Asparagus officinalis* L.
Bitter bean/Sataw	Sa-to (สะตอ)	*Parkia speciosa* Hassk.
Bitter melon	Ma-ra (มะระ)	*Momordica charantia* L.
Carrot	Car-rot (แครอท)	*Daucus carota* L.
Chinese chive	Kui-chai (กุยช่าย)	*Allium tuberosum* Roxb.
Common Asiatic weed	Ma-khuea-pro (มะเขือเปราะ)	*Linociera parkinsonii* Hutch.
Eggplant/Brinjal	Ma-khuea-phuang (มะเขือพวง)	*Solanum torvum* Sw.
Garden peas	Thua-lan-tao (ถั่วลันเตา)	*Pisum satavim* L.
Horse-tamarind/White Popinac/Lead Tree	Kra-thin (กระถิน)	*Leucaena leucocephala* (Lamk.) de Wit
Lady's finger/ Okra	Kra-chiap-mon (กระเจี๊ยบมอญ)	*Abelmoschus esculentus* (L.) Moench
Leek	Kra-thiam-ton (กระเทียมต้น)	*Allium porrum* Linn.
Pumpkin	Fak-thong (ฟักทอง)	*Cucurbita moschata* Decne.
Radish	Hua-phak-kat-khao (หัวผักกาดขาว)	*Raphanus sativus* L.

Table 6. List of studied foods (common name, Thai name and scientific name) (continued)

Common name	Thai name	Scientific name
Fruits		
Banana, common, ripe	Kluai-nam-wa (กล้วยน้ำว้าสุก)	*Musa sapientum* L.
Banana, common, unripe	Kluai-nam-wa (กล้วยน้ำว้า)	*Musa sapientum* L.
Banana, dessert, Valery variety	Kluai-hom (กล้วยหอม)	*Musa sapientum* L.
Banana, rice	Kluai-khai (กล้วยไข่)	*Musa suerier* L.
Banana, silver Bluggoe	Kluai-hak-muk (กล้วยหักมุก)	*Musa sapientum* L.
Dragon fruits, red	Kaew-mang-gon, dang (แก้วมังกรแดง)	*Hylocereus undatus* (Haw.) Britton& Rose
Dragon fruits, white	Kaew-mang-gon, khaw (แก้วมังกรขาว)	*Hylocereus undatus* (Haw.) Britton & Rose
Spices		
Chinese deys	Kra-chai (กระชาย)	*Boesenbergia pundurata* (Roxb.) Schitr
Garlic, common	Kra-thiam (กระเทียมไทย)	*Allium sativum* L.
Garlic, Chinese	Kra-thiam-chin (กระเทียมจีน)	*Allium chinense* G. Don
Garlic, great headed/ Garlic, elephant	Kra-thiam-thon-hua-yai (กระเทียมโทนหัวใหญ่)	*Allium ampeloplasum* Linn.
Galangal	Kha (ข่า)	*Languas galanga* Sw.
Ginger	Khing (ขิง)	*Zingiber officinale* Roscoe
Onion	Hom-yai (หอมใหญ่)	*Allium cepa* L.
Onion, red	Hom-khaek (หอมแขก)	*Allium atrorubens* S. Wats.
Shallot	Hom-daeng (หอมแดง)	*Allium ascalonicum* L.
Turmeric	Kha-min (ขมิ้นแดง)	*Curcuma longa* L.
Turmeric, white	Kha-min kao (ขมิ้นขาว)	*Curcuma mangga* val amp zijp
Seeds and nuts		
Hairy basil seed (Psyllium seed husks)	Mang-lak (เม็ดแมงลัก)	*Ocimum americanum* Linn.
Jackfruit, seed, boiled	Ma-led-Kha-noon (เม็ดขนุน, ต้ม)	*Artocarpus heterophyllus* Lam.
Job's tear, seed	Dueai (ลูกเดือย)	*Coix lachryma-jobi*, L.
Sunflower seed	Ma-led-Tan-ta-wan (เมล็ดทานตะวัน)	*Helianthus annuus* Linn.

4.2 Sample preparation

Fresh samples, 0.5-1 kg each, of the selected foods were randomly purchased from three retailed shops at Salaya market (showed in **Table 6**). All food samples were kept in plastic bags and immediately transferred to the laboratory at the Institute of Nutrition, Mahidol University (INMU). Single composite samples of each food from three retailers were prepared (*n*=1). The samples except seeds and nuts were washed with tap water until clean, blotted to remove excess water, weighed and discarded the inedible portion (recorded % edible portion), rinsed with deionised water once, blotted with clean tissue paper and then air dried. The remaining edible portion was cut into small size and homogenised by food processor (Tefal® Kaleo Blender, France). The homogenised samples were divided into 2 portions; one portion was determined as fresh sample for moisture analysis on the same day of sample preparation and another portion was dried by a freeze-dryer (Lyovac®, Germany). The freeze-dried samples were homogenised by a grinder (Panasonic® MX-210GN, Japan) to obtain fine powder. Weight of sample before and after freeze drying was used for calculation of inulin, FOS and sugar contents in fresh samples. Seeds and nuts were ground by Panasonic® grinder and analysed for moisture. Each sample was kept in an acid-washed screwed-cap polyethylene bottle until analysis. The single composite samples of each food were analysed to identify potential sources of inulin-type fructans and fructo-oligosaccharide. Two more sets of potential food sources from the preliminary study were processed and prepared (*n*=3) as described in test samples of Salaya market.

4.3 Moisture determination

Moisture in fresh samples was determined according to the method of AOAC (2005, 990.19) [66] in which 1-2 g of homogenised sample was dried in an oven at 100±5ºC until constant weight. Moisture content as g per 100g sample was then calculated from the weight loss. The determination was performed in duplicate analysis. The procedure is shown in **APPENDIX B**. The moisture content were used for calculation of total solid (%total solid = 100-%moisture) and express the amount of inulin-type fructans and FOS content on a dry matter and also used for comparison with other studies.

4.4 Inulin-type fructans and fructo-oligosaccharide determination

Based on the AOAC (2005, 997.08) method [66], total sugar, fructans and FOS were extracted with hot water. Inulin-type fructans and FOS in a portion of the hot water extract were hydrolysed by inulinase. Both hot water extracted and enzyme hydrolysed fractions were derivatised with oxymation and silyltion reaction and then individual sugar were determined by gas chromatography. The procedure of the inulin-type fructans analysis is presented in **APPENDIX C**. The determination was performed in duplicate analysis.

4.4.1 Extraction of sugar and inulin

Freeze-dried sample (1-5 g) which contained about 1 g of inulin-type fructans, was weighed into a 100 mL screwed cap bottle and extracted with about 50 mL of hot water. The pH of solution was adjusted to 6.5-8.0 with 0.05 N KOH or 0.05 N HCl. Final volume was made up to 50 mL with deionised water. The mixture was incubated at 85±2ºC in shaking water-bath for 15 min.

4.4.2 Analysis of sugar in hot water extract (without hydrolysis)

Extracted sample solution (3-5 g) in **Section 4.4.1** was weighed directly into a 25 mL volumetric flask which contained 0.01 g of rhamnose as an internal standard and then diluted to 25 mL with deionised water. The solution was used for derivatisation step (**Section 4.4.5**).

4.4.3 Enzyme hydrolysis of inulin

About 15 g of extracted sample solution in **Section 4.4.1** was weighed into a 100 mL screwed cap bottle. Equal amount of acetate buffer was added. The pH of mixture solution was adjusted to 4.5±0.5 with 0.2 M acetic acid or 0.2 M Na-acetate. Sufficient amount of inulinase (i.e. 400 µL of inulinase (Sigma-Aldrich®, USA) which has enzyme activity of 1740 INU/G) was added and incubated for 30 min in shaking water bath, at 60±2 ºC. After cooling, 5 g of sample solution after hydrolysis was transferred into a 25 mL volumetric flask which contained 0.01 g of rhamnose as an internal standard. The volume of the mixture was made up to 25 mL with deionised water. The final solution was used for derivatisation step (**Section 4.4.5**).

4.4.4 Standard preparation

Each standard sugar was prepared in appropriate concentrations of four levels from 0.2 to 0.8 mg/mL of fructose, glucose and sucrose (Fluka®, USA) and 0.2 to 1.0 mg/mL for GF_2 (1-kestose), GF_3 (nystose) and GF_4 (1^F- β fructofuranosylnystose) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) which contained 0.01 g of rhamnose (Sigma®, USA) as an internal standard. The final solutions were used for derivatisation step (**Section 4.4.5**).

4.4.5 Sugar derivatisation

Solution of extracted samples and standards from **Section 4.4.2 to 4.4.4** was pipetted (200 µL) into a set of 10 mL screwed-cap tubes. They were dried in a vacuum desiccator until dryness (approximately 3 h). Two steps of sugar derivatisation (oximation and trimethysilylation) were carried out as follow; 1) 400 µL of hydroxylamine chloride in pyridine was added into each tube; mixed the solution for 10 min on vortex mixer; incubated at 60 ºC for 5 min in a water-bath; then centrifuged at 2000 rpm for 5 min; 2) 200 µL of silylating agent (tri-methysilylimidazole, TSIM) was added into each tube, shaked again for 10 min on vortex mixer and neutralised the excess of TSIM with 1000 µL of deionised water. A 2000 µL of isooctane was added and shaked again for 3 min. After complete reaction, the mixture was centrifuged at 2000 rpm for 5 min. Finally, the upper layer (isooctane) was transferred into a gas chromatography (GC) vial and then sugars were determined by GC. The schematic diagram of sampling, sample preparation, inulin-type fructans and sugar analysis is shown in **Figure 4**.

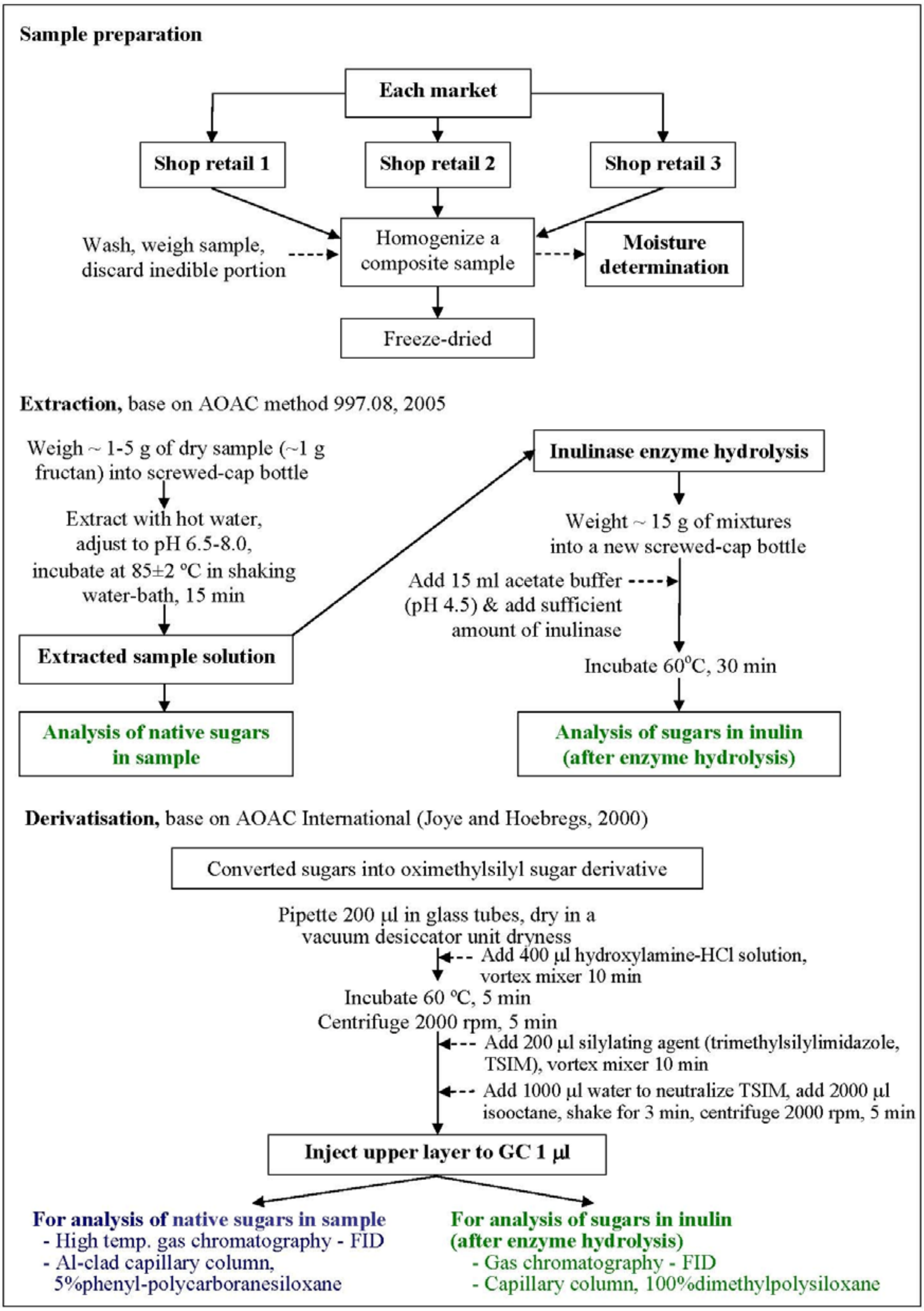

Figure 4. Schematic diagram of inulin-type fructans and sugar analysis

4.4.6 Sugar determination by gas chromatography

Sugar in the hot water extract which contained free fructose, glucose and sucrose and FOS (GF_2, GF_3, GF_4) and in the corresponding standard solutions were determined by high temperature GC (Agilent® 7890, USA), equipped with a capillary aluminum-clad column (5%-phenyl-polycarboranesiloxane, HT-5, Restek®, USA). The temperature of flame ionization detector (FID) was set at 447ºC. Helium gas was used as carrier gas, set at a flow rate of 9 mL/min. Nitrogen gas was used as a make up gas, set at flow rate of 25 mL/min. Air and hydrogen gas for FID detector were set at 400 mL/min and 40 mL/min, respectively. For sugar in the solution of inulinase hydrolysed extract which contain hydrolysed sugar, sugar fractions from inulin and FOS (fructose and glucose) were determined by another set of GC (Agilent® 6850, USA) which equipped with 100% dimethylpolysiloxane (HP-1, Agilent® J&W, USA). The temperature of FID detector was set at 350ºC. The same carrier and make up gases were used; they were set at flow rate of 2 mL/min and 30 mL/min, respectively. Air and hydrogen gas for FID detector were set at 350 mL/min and 35 mL/min, respectively.

4.5 Analytical quality control

A commercial mixture of oligofructose (Orafti® P95) was used as a quality control sample for FOS and sugar determination. Milk powder, fortified with inulin (Protection 1+, Nestle®), was used for inulin-type fructans and some sugars (fructose, glucose and sucrose). Assigned value of FOS in the commercial oligofructose, provided by Orafti, Co., Ltd, Belgium, was 93.2 g/100 g whereas the developed assigned value, as mean±SD, by Orafti and by the Institute of Nutrition, Mahidol University (INMU) laboratories, with N=10, was 89.09±1.72 g/100 g. Assigned values of inulin and sugars (sum of fructose, glucose and sucrose) in the fortified milk powder, developed by the same laboratories, were 2.79±0.2 and 2.17±0.22 g/100g, respectively. For daily quality control system, both materials were analysed in each set along with unknown samples. The accepted values of the results must fall into the mean±2SD of the assigned value of each component which indicates a good performance of the analyst. Precision of each analysis, which indicates by the percent difference between duplicate determinations, must be less than 10. Percent recovery of FOS was also conducted using the commercial mixture of oligofructose from Orafti. The accepted value of FOS recovery was 100$\pm$5 %.

4.6 Calculation and expression of results

Amount of inulin-type fructans was calculated from the difference of the amounts of each sugars – fructose, glucose and sucrose – before and after enzyme hydrolysis as given in AOAC (2005, 997.08). Individual fructo-oligosacharide (FOS) in the hot water extract were determined and quantified from each calibration curve of GF_2, GF_3 and GF_4. Detail of equation and calculation of inulin-type fructan is shown in **APPENDIX C**.

4.7 Statistical analysis

Content of moisture, inulin-type fructans, fructo-oligosaccharides (FOS) (sum of GF_2, GF_3 and GF_4) and sugars (sum of fructose, glucose and sucrose) were presented in terms of mean value $\pm$ standard deviation (SD) using Microsoft Office Excel 2003.

CHAPTER V
RESULTS

The result of this study are divided into 2 parts: 1) inulin-type fructans, fructo-oligosaccharide (FOS) and sugars in selected Thai foods - preliminary study and 2) levels of the components in potential food sources.

5.1 Analytical quality control

The gas chromatography technique, both high temperature GC (Agilent® 7890) and GC (Agilent® 6850), provided good repeatability (*r*) (RSD_r<2%, n=5) and reproducibility (*R*) (RSD_R<5%, n=37) for inulin determination. Good accuracy was also demonstrated when standard inulin powder (Orafti® P95, Belgium) was analysed, showing percent recovery of 98.3±2.9% (n=18). On analysis of inulin in the QC sample in each set of unknown samples, the value obtained fell within the mean±2SD of the assigned value (2.8±0.1 g inulin/100g fresh weight).

5.2 Inulin-type fructans, fructo-oligosaccharides and sugars in selected Thai plant foods - preliminary study

5.2.1 Edible portion and moisture content

Table 7 shows edible portion (%) and moisture content (g/100g) of the selected foods each as a single composite sample of three individual test items collected from one representative market. The average edible portion of these foods ranged from 30 to 100% and the moisture content varied from 2.27 to 95.36 g/100g.

In fresh starchy roots and tubers, the edible portion ranged from 72% in small taro to 93% in sweet potato. Moisture contents ranged from 63.31 g/100g in red variety of sweet potato to 95.36 g/100g in Noh kala shoot. One exception was found in dried yam which was purchased as dried and completely peeled 100 % edible portion and which contained the lowest moisture content of 14.97 g/100g.

The minimum of average edible portion (less than 50%) was found in fresh vegetables such as horse-tamarind, bitter bean and leek (42%, 32% and 30%, respectively). Other selected foods of this group ranged from 66% in asparagus to 93% in garden peas. Moisture content ranged from 69.33 g/100g in bitter bean to 95.00 g/100g in bitter cucumber.

In various species of banana (dessert, common, silver Bluggoe and rice varieties), the edible portion ranged from 62.71% to 74.81% and moisture content ranged from 64.69 to 73.62 g/100g. Highest moisture content was found in banana-rice variety (73.62 g/100g) whereas the lowest moisture content was found in ripe banana-common variety (64.69 g/100g). The edible portion of dragon fruits ranged between 68.23% in white-dragon fruit and 73.49% in red-dragon fruit. Both varieties of dragon fruits contained a higher moisture content, about 85.5 g/100g.

Average edible portion of fresh spices ranged between 63% in Chinese deys and 96% in onion, except that of white turmeric (100%) which was purchased as a complete peel. The moisture content of this group ranged from 62.10 to 93.92 g/100g. Great headed garlic contained lowest moisture content (62.10 g/100g) whereas white-turmeric contained highest moisture content (93.92 g/100g).

The edible portion of the raw seed and nut group, which is consumed as a whole without peeling, was 100%, except those of boiled jackfruit and sunflower seed which were found to be 96% and 45%, respectively. Lowest moisture content was found in this food group (less than 8.7%), ranging from the minimum of 3.42 g/100g in sunflower seed to 8.66 g/100g in Job’s tear seed. Boiled jack fruit seed contained a higher level of moisture, 52.34 g/100g.

Table 7. Edible portion and moisture content of studied foods - preliminary study

Common name	Edible portion[(1)] (%)	Moisture[(2)] (g/100g)
Starchy roots and Tubers		
Cassava	75.77	64.99
Jerusalem artichoke	78.98	73.34
Noh kala shoot	73.09	95.36
Potato	89.12	82.34
Sweet potato	92.88	71.83
Sweet potato, red	80.54	63.31
Sweet potato, white	80.47	65.02
Sweet potato, yellow	90.09	72.28
Taro, hom variety	85.46	74.43
Taro, small size	71.50	67.26
Yam bean	90.36	93.73
Yam, dried	100[(3)]	14.97
Vegetables		
Asparagus	66.18	93.90
Bitter bean/Sataw	31.89	69.33
Bitter melon	85.87	95.00
Carrot	86.72	88.97
Chinese chive	85.85	87.41
Common Asiatic weed	89.17	78.64
Eggplant/Brinjal	92.30	90.75
Garden peas	92.73	89.47
Horse-tamarind/White Popinac/ Lead Tree	41.16	76.50
Lady's finger/ Okra	88.37	88.95
Leek	29.65	89.68
Pumpkin	87.29	86.93
Radish	78.84	94.11
Fruits		
Banana, common, ripe	74.81	64.69
Banana, common, unripe	71.97	67.26
Banana, dessert, Valery variety	66.84	71.08
Banana, rice	74.39	73.62
Banana, silver Bluggoe	62.71	68.26
Dragon fruit, red	73.49	85.58
Dragon fruit, white	68.23	85.44

Table 7. Edible portion and moisture content of studied foods - preliminary study (continued)

Common name	Edible portion[1] (%)	Moisture[2] (g/100g)
Spices		
Chinese deys	62.63	92.52
Garlic, common	85.19	65.38
Garlic, Chinese	89.39	70.66
Garlic, great headed/ Garlic, elephant	87.46	62.10
Galangal	81.00	85.14
Ginger	81.66	90.88
Onion	95.59	92.75
Onion, red	90.02	86.72
Shallot	86.98	86.62
Turmeric	66.18	90.02
Turmeric, white	100[3]	93.92
Seeds and nuts		
Hairy basil seed (Psyllium seed husks)	100	8.49
Jackfruit, seed, boiled	96.32	52.34
Job's tear, seed	100	8.66
Sunflower seed	45.09	3.42

[1] Each values calculated from edible portions of three single samples collected from one market

[2] Each average values obtained from duplicate analysis of a single composite sample (derived from three individuals) collected from one market

[3] Purchased as edible part of complete peel

5.2.2 Inulin-type fructans and fructo-oligosaccharide content in various plant foods

The amount of fructan in the studied samples was determined as the amounts of individual sugars (fructose, glucose and sucrose) after hydrolysis with inulinase enzyme treatment minus the amounts of the existing sugars in the original sample. The components of fructo-oligosaccharide (FOS) were analysed as 1-kestose (GF_2), nystose (GF_3) and 1F-β-fructofuranosylnystose (GF_4). An example of a chromatogram for analysis of sugars (fructose, glucose and sucrose) and FOS (GF_2, GF_3 and GF_4) is shown in **APPENDIX D**.

The amounts of inulin-type fructans and fructo-oligosaccharide (as GF_2, GF_3 and GF_4), as g/100g of fresh sample (FW), in 47 selected Thai foods collected as three single samples from one market for preliminary study are presented in **Tables 8-12.** Inulin content in all studied foods ranged from not detected (less than limit of detection, 0.01 g/100g) to 25.7 g/100g FW. More detailed information of the components in each food group is shown as follow.

5.2.2.1 Starchy roots and tubers

As shown in **Table 8,** most of the studied fresh starchy roots and tubers contained small amounts of inulin (less than 0.52 g/100g FW), except for Jerusalem artichoke or Kaen-ta-wan which contained the highest level of inulin (19.42 g/100g FW). None or less than detectable amount of inulin (< 0.01 g/100g) was found in potato, taro and dried yam.

Among all foods studied, Jerusalem artichoke or Kaen-ta-wan contained the highest amount of FOS (sum of GF_2, GF_3, and GF_4), 5.16 g/100g FW. Other starchy roots and tubers contained not detectable to low amounts (<0.2 g/100g FW) of FOS.

5.2.2.2 Vegetables

Table 9 shows the inulin and fructo-oligosaccharide (FOS) contents in the vegetable group**.** Fresh vegetables contained small amounts of inulin, which ranged from undetectable amount in eggplant, common Asiatic weed and carrot to 0.62 g/100g FW in garden pea.

Similar findings were demonstrated for FOS, the content varied from undetectable to maximum of 0.23 g/100g FW in both bitter bean and leek.

Table 8. Inulin-type fructans and fructo-oligosaccharide content (g/100g of fresh sample, FW) of fresh starchy roots and tubers - preliminary study

Common name	Fructo-oligosaccharide (FOS)[1] (g/100g FW)				Inulin-type fructans[1] (g/100g FW)
	GF_2	GF_3	GF_4	Sum[2]	
Starchy roots and Tubers					
Cassava	0	0	0	0	0.36
Jerusalem artichoke	2.02	1.75	1.39	5.16	19.42
Noh Kala shoot	0	0	0	0	0.52
Potato	0	0	0	0	ND
Sweet potato	0.14	0	0	0.14	0.27
Sweet potato, red	0.19	0	0	0.19	0.19
Sweet potato, white	0.19	0	0	0.19	0.05
Sweet potato, yellow	0.11	0	0	0.11	0.13
Taro, hom variety	0	0	0	0	ND
Taro, small size	0.13	0	0	0.13	ND
Yam bean	0.03	0	0	0	0.06
Yam, dried	0.06	0.07	0.08	0.21	ND

(1) Value obtained from duplicate analysis of a single composite sample collected from one market

(2) Sum of individual fructo-oligosaccharide (GF_1, GF_2, and GF_3); GF_2=1-kestose (1-ketotriose), GF_3=nystose (1,1-ketotetraose), GF_4=fructosylnystose (1,1,1-ketopentaose)

ND= not detected (< 0.01 g/100g)

Table 9. Inulin-type fructans and fructo-oligosaccharide content (g/100g of fresh sample, FW) of fresh vegetables - preliminary study

Common name	Fructo-oligosaccharide (FOS)(1) (g/100g FW)				Inulin-type fructan(1) (g/100g FW)
	GF_2	GF_3	GF_4	Sum(2)	
Vegetables					
Asparagus	0	0	0	0	0.02
Bitter bean/Sataw	0.23	0	0	0.23	0.36
Bitter cucumber	0	0	0	0	0.05
Carrot	0.04	0	0	0.04	ND
Chinese chive	0.02	0.01	0.01	0.03	0.06
Common Asiatic weed	0	0	0	0	ND
Eggplant	0	0	0	0	ND
Garden peas	0.09	0	0	0.09	0.62
Horse-tamarind	0	0	0	0	0.21
Lady's finger, Okra	0	0	0	0	0.03
Leek	0.11	0.06	0.06	0.23	0.48
Pumpkin/Cushaw	0	0	0	0	0.03
Radish	0	0	0	0	0.15

(1) Value obtained from duplicate analysis of a single composite sample collected from one market

(2) Sum of individual fructo-oligosaccharide (GF_1, GF_2, and GF_3); GF_2=1-kestose (1-ketotriose), GF_3=nystose (1,1-ketotetraose), GF_4=fructosylnystose (1,1,1-ketopentaose)

ND= not detected (< 0.01 g/100g)

5.2.2.3 Fruits

A low level of inulin (<0.5 g/100g FW) was found in different varieties of bananas and dragon fruits (**Table 10**). No inulin was detected in dessert and silver Bluggoe varieties of banana. The level in unripe banana, common variety (0.06 g/100g FW), was lower than in ripe banana (0.040 g/100g FW).

A low level of fructo-oligosaccharide was also found in the fruit group ranging from not detected to 0.09 g/100g FW in common variety of ripe banana.

Table 10. Inulin-type fructans and fructo-oligosaccharide content (g/100g of fresh sample, FW) of fruits - preliminary study

Common name	Fructo-oligosaccharide (FOS)(1) (g/100g FW)				Inulin-type fructan(1) (g/100g FW)
	GF_2	GF_3	GF_4	Sum(2)	
Fruits					
Banana, common, ripe	0.09	0	0	0.09	0.40
Banana, common, unripe	002	0	0	0.02	0.06
Banana, dessert, Valery variety, unripe	0	0	0	0	ND
Banana, rice, half–ripe	0.04	0	0	0.04	0.34
Banana, silver Bluggoe, unripe	0.02	0.01	0	0.03	ND
Dragon fruit, red	0	0	0	0	0.21
Dragon fruit, white	0	0	0	0	0.15

(1) Value obtained from duplicate analysis of a single composite sample collected from one market

(2) Sum of individual fructo-oligosaccharide (GF_1, GF_2, and GF_3); GF_2=1-kestose (1-ketotriose), GF_3=nystose (1,1-ketotetraose), GF_4=fructosylnystose (1,1,1-ketopentaose)

ND= not detected (< 0.01 g/100g)

5.2.2.4 Spices

A high amount of inulin and fructo-oligosaccharide (GF_2, GF_3 and GF_4) was found in some fresh spices, i.e., common garlic (23.01 g/100g FW), great headed garlic (24.04 g/100g FW) and Chinese garlic (25.65 g/100g FW), shown in **Table 11.** Red onion and shallot contained a medium amount of inulin, 2.49-9.39 g/100g FW. Low inulin content, 0.02-0.44 g/100g FW, was found in onion, turmeric, white-turmeric, and Chinese deys. Undetectable inulin (less than 0.01 g/100g FW) was found in galangal and ginger.

The fructo-oligosaccharide content in fresh spices ranged from not detected level to 5.34 g/100g FW. Shallot, great headed garlic and red onion provided a high amount of FOS (5.34, 3.27 and 2.47 g/100g FW, respectively). Low FOS content was found in onion, Chinese garlic, common garlic and turmeric (0.03 - 0.96 g/100g FW).

Table 11. Inulin-type fructans and fructo-oligosaccharide content (g/100g of fresh sample, FW) of fresh spices - preliminary study

Common name	Fructo-oligosaccharide (FOS)(1) (g/100g FW)				Inulin-type fructans(1) (g/100g FW)
	GF_2	GF_3	GF_4	Sum(2)	
Spices					
Chinese deys	0	0	0	0	0.04
Garlic, common	0.34	0.32	0.30	0.96	23.01
Garlic, Chinese	0.28	0.20	0.16	0.64	25.65
Garlic, great headed / Garlic, elephant	1.00	1.09	1.18	3.27	24.04
Galangal	0	0	0	0	ND
Ginger	0	0	0	0	ND
Onion	0.22	0	0	0.22	0.44
Onion, red	0.97	0.80	0.70	2.47	2.49
Shallot	1.63	1.81	1.90	5.34	9.39
Turmeric	0.03	0	0	0.03	0.13
Turmeric, white	0	0	0	0	0.02

(1) Value obtained from duplicate analysis of a single composite sample collected from one market

(2) Sum of individual fructo-oligosaccharide (GF_1, GF_2, and GF_3); GF_2=1-kestose (1-ketotriose), GF_3=nystose (1,1-ketotetraose), GF_4=fructosylnystose (1,1,1-ketopentaose)

ND= not detected (< 0.01 g/100g)

5.2.2.5 Seeds and nuts

Inulin and fructo-oligosaccharide contents in raw seeds and nuts are presented in **Table 12.** Among the studied seeds and nuts, low levels of inulin and FOS (with GF_2 as the main FOS fraction) were found only in sunflower seed, 0.34 and 1.75 g/100g FW, respectively.

Table 12. Inulin-type fructans and fructo-oligosaccharide content (g/100g of fresh sample, FW) of raw seeds and nuts - preliminary study

Common name	Fructo-oligosaccharide (FOS)[1] (g/100g FW)				Inulin-type fructans[1] (g/100g FW)
	GF_2	GF_3	GF_4	Sum[2]	
Seeds and nuts					
Hairy basil seed (Psyllium seed husks)	0	0	0	0	ND
Jackfruit, seed, boiled	0	0	0	0	ND
Job's tear, seed	0	0	0	0	ND
Sunflower seed	1.68	0.07	0	1.75	0.34

(1) Value obtained from duplicate analysis of a single composite sample collected from one market

(2) Sum of individual fructo-oligosaccharide (GF_1, GF_2, and GF_3); GF_2=1-kestose (1-ketotriose), GF_3=nystose (1,1-ketotetraose), GF_4=fructosylnystose (1,1,1-ketopentaose)

ND= not detected (< 0.01 g/100g)

Considering the distribution of FOS (GF_2, GF_3 and GF_4) fractions in all studied food groups, it was found that GF_2 (1-kestose) seems to be the main fraction of FOS except in some spices, i.e. garlic, red onion and shallot, and some starchy roots and tubers, i.e., dried yam and Jerusalem artichoke which showed similar ratio of GF_2, GF_3 and GF_4.

Figures 5 and 6 present the levels of inulin and FOS in various food groups in a descending order. The descending orders of the levels of inulin for the top 6 foods are Chinese garlic > great headed garlic > garlic > Jerusalem artichoke > shallot > red onion whereas that of FOS for the top four are shallot > Jerusalem artichoke > great headed garlic > red onion.

The presentation assists in selection of potential food sources of required components for further study.

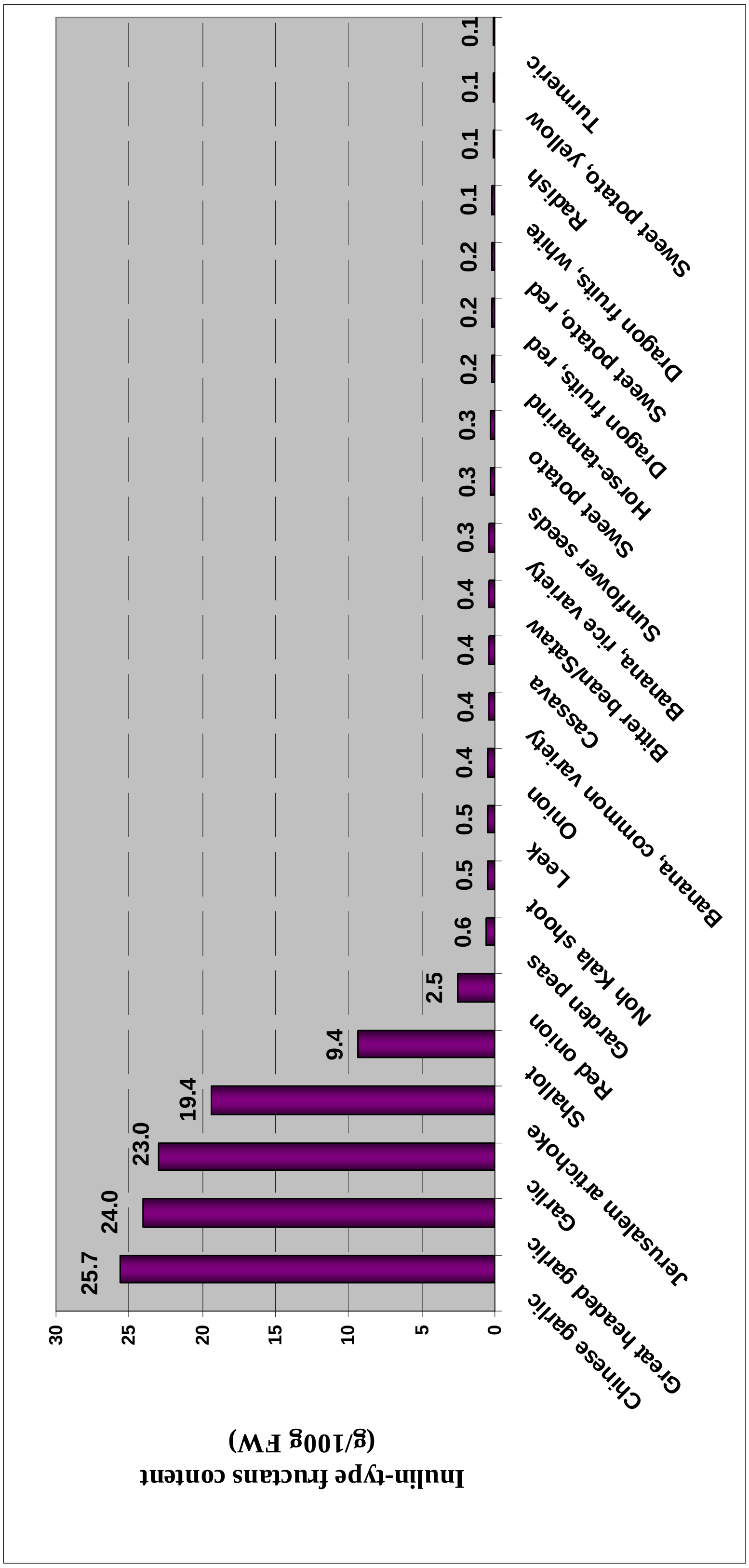

Figure 5. Inulin-type fructans content (g/100g on fresh weight) of some selected foods - preliminary study

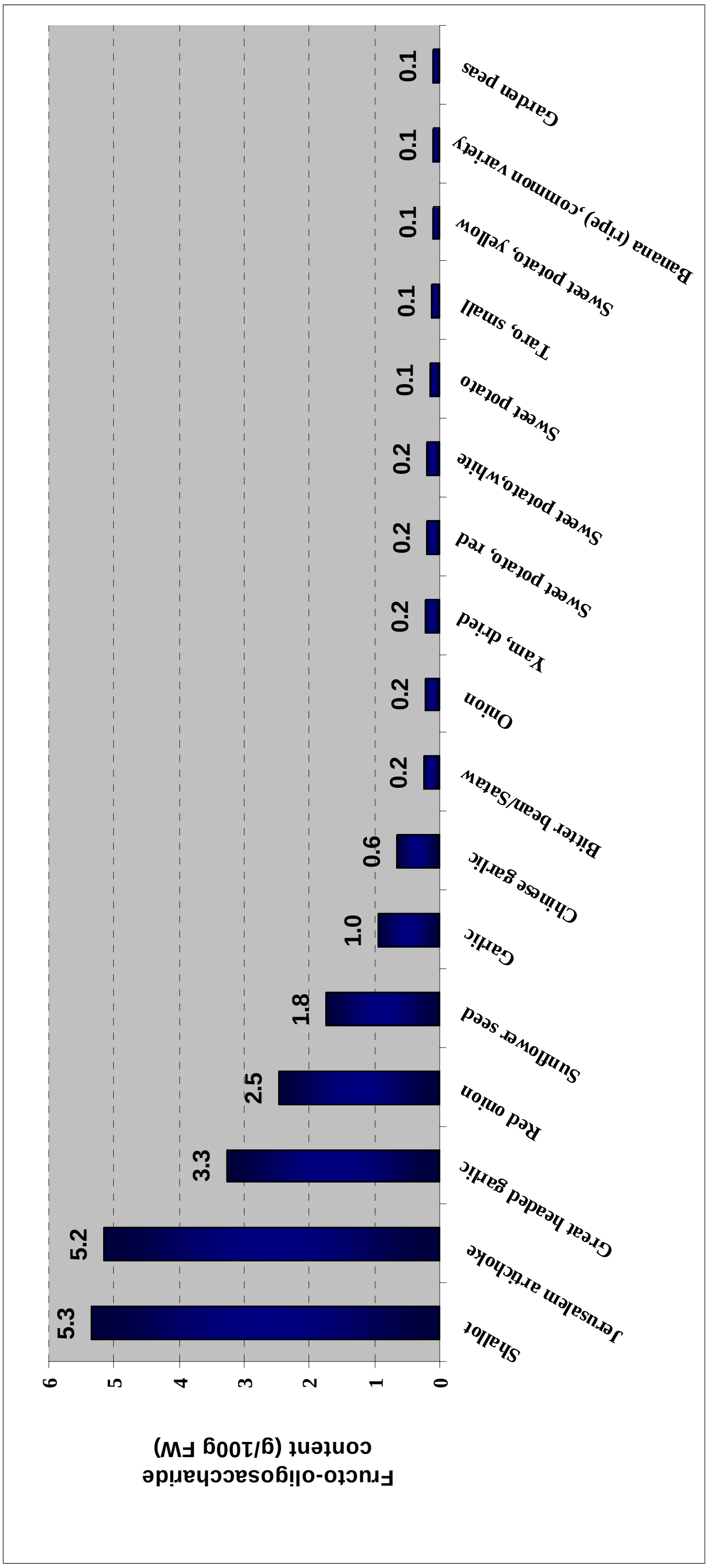

Figure 6. Fructo-oligosaccharide content (g/100g on fresh weight) of some selected foods - preliminary study

5.2.3 Sugar content in various plant foods

Sugar content, determined as fructose, glucose and sucrose, in various foods collected from one market and prepared as single composite samples for preliminary study are presented in **Tables 13-17**. The amount of total sugar (sum of fructose, glucose and sucrose) in most fresh foods was less than 4.5 g/100g FW, except a higher level was found in carrot, ripe banana and dragon fruit (6.65-10.51 g/100 g FW).

5.2.3.1 Starchy roots and tubers

As shown in **Table 13**, sugar content (sum of fructose, glucose and sucrose) in fresh starchy roots and tubers ranged from 0.71 to 4.26 g/100g FW. The highest sugar content was found in yellow variety of sweet potato. Sucrose was found to be the main sugar in all starchy roots and tubers**,** except in Noh kala shoot and yam bean which contained fructose and glucose as the main fractions.

Table 13. Sugar content (g/100g fresh weight, FW) in starchy roots and tubers - preliminary study

Common name	Sugar (g/100g FW)[(1)]			
	Fructose	**Glucose**	**Sucrose**	**Sum**
Starchy roots and tubers				
Cassava	0.27	0.16	1.74	2.17
Jerusalem artichoke	0.21	0.06	1.15	1.41
Noh Kala shoot	1.10	1.19	0.17	2.46
Potato	0.93	1.02	1.46	3.42
Sweet potato	1.22	0.43	1.64	3.29
Sweet potato, red	0.11	0.02	2.57	2.70
Sweet potato, white	0.93	0.43	1.72	3.08
Sweet potato, yellow	1.04	1.17	2.05	4.26
Taro, hom variety	0.18	0.00	0.77	0.95
Taro, small size	0.31	0.38	2.33	3.02
Yam bean	1.35	1.98	0.70	4.02
Yam, dried	0.22	0.14	0.35	0.71

[(1)] Each value obtained from duplicate analysis of a single composite sample, collected from one market

5.2.3.2 Vegetables

Values of sugar in fresh vegetables, ranging from 0.12 (horse-tamarind) to 6.65 g/100g FW (carrot), are shown in **Table 14**. Most of studied vegetables contained low levels of individual of sugar (less than 2 g/100g FW), except that of carrot which contained the highest amount of sucrose (5.45 g/100g FW).

Table 14. Sugar content (g/100g fresh weight, FW) in fresh vegetables - preliminary study

Common name	Sugar (g/100g FW)[(1)]			
	Fructose	Glucose	Sucrose	Sum
Vegetables				
Asparagus	0.73	0.42	0.01	1.16
Bitter bean/Sataw	0.01	0.03	1.46	1.50
Bitter cucumber	0.74	1.54	0	2.28
Carrot	0.84	0.36	5.45	6.65
Common Asiatic weed	0.10	0.30	0.08	0.47
Chinese chive	1.17	0.13	0.05	1.36
Eggplant	0.67	0.68	0.15	1.51
Garden peas	0.14	0.47	0.98	1.60
Horse-tamarind	0	0.12	0.01	0.12
Lady's finger, Okra	0.89	0.44	0.68	2.01
Leek	1.95	1.73	0	3.68
Pumpkin/Cushaw	1.34	0.56	1.61	3.51
Radish	1.97	1.58	0.07	3.62

(1) Each value obtained from duplicate analysis of a single composite sample, collected from one market

5.2.3.3 Fruits

As shown in **Table 15**, sugars (sum of fructose, glucose and sucrose) in banana and dragon fruits ranged from 3.56 to 10.51 g/100g FW. Unripe banana, dessert variety, contained an exceptionally low level of sugar, 0.26 g/100g FW. High sugar levels (7-10 g/100g FW) were found in ripe and half-ripe bananas (common and rice variety) and dragon fruits.

Fructose and glucose were the main sugars found in ripe and unripe common bananas and in dragon fruits whereas sucrose was the main sugar found in rice and silver Bluggoe varieties of bananas.

Table 15. Sugar content (g/100g fresh weight, FW) in fruits - preliminary study

Common name	Sugar (g/100g FW)[1]			
	Fructose	**Glucose**	**Sucrose**	**Sum**
Fruits				
Banana, common, ripe	4.90	4.50	0.67	10.07
Banana, common, unripe	2.64	2.75	1.34	6.73
Banana, dessert, Valery variety, unripe	0.17	0.09	0	0.26
Banana, rice, half-ripe	1.71	1.28	7.53	10.51
Banana, silver Bluggoe, unripe	0.21	0.35	3.00	3.56
Dragon fruit, red	2.78	4.30	0	7.09
Dragon fruit, white	2.63	4.78	0	7.41

(1) Each value obtained from duplicate analysis of a single composite sample, collected from one market

5.2.3.4 Spices

As shown in **Table 16,** most of fresh spices contained low sugar levels (less than 2 g/100g FW), ranging from 0.5 to 2.17 g/100g FW, except that of onion which contained a higher level (4.06 g/100g FW). No specific pattern of individual sugars was obtained in this food group. Individual sugars (fructose, glucose and sucrose) were found in low levels (less than 1.76 g/100g FW).

5.2.3.5 Seeds and nuts

Low levels of sugar (<1 g/100g FW) were found in selected seeds and nuts (**Table 17**) except a higher level of 2.38 g/100g was found in sunflower seeds with sucrose as the main sugar.

Table 16. Sugar content (g/100g fresh weight, FW) in spices - preliminary study

Common name	Sugar (g/100g FW)[1]			
	Fructose	Glucose	Sucrose	Sum
Spices				
Chinese deys	0.21	0.22	0.07	0.50
Garlic, common	0.12	0.08	0.99	1.19
Garlic, Chinese	0.09	0.04	0.68	0.81
Garlic, great headed	0.18	0.07	1.76	2.02
Galangal	0.90	1.11	0.16	2.17
Ginger	0.39	0.37	0.13	0.90
Onion	1.57	1.06	1.43	4.06
Onion, red	0.51	0.65	0.87	2.04
Shallot	0.35	0.18	1.36	1.88
Turmeric	0.50	0.53	0.65	1.68
Turmeric, white	0.60	0.81	0.09	1.49

[1] Each value obtained from duplicate analysis of a single composite sample, collected from one market

Table 17. Sugar content (g/100g fresh weight, FW) in seeds and nuts - preliminary study

Common name	Sugar (g/100g FW)[1]			
	Fructose	Glucose	Sucrose	Sum
Seeds and nuts				
Hairy basil seed (Psyllium seed husks)	0.13	0.11	0.27	0.51
Jack fruit, seed, boiled	0.09	0.06	0.15	0.30
Job's tear, seed	0.05	0.08	0.61	0.74
Sunflower seed	0.05	0.12	2.21	2.38

[1] Each value obtained from duplicate analysis of a single composite sample, collected from one market

5.3 Inulin-type fructans, fructo-oligosaccharides and sugars in selected Thai plant foods - potential food sources

As mentioned earlier, forty-seven various Thai foods, distributed in five food groups, were selected from one market (three individuals each) for screening of potential sources of inulin and fructo-oligosaccharide (FOS) as a preliminary study. As shown in **Figures 5 and 6,** those which contained inulin and FOS at the level of more than 2 and 0.5 g/100g FW, respectively, were considered as potential sources of inulin and FOS. They were varieties of garlic, Jerusalem artichoke, red onion and shallot. Triplicate samples of these foods were collected from two more markets and their single component samples were prepared. The results of edible portion, moisture, inulin-type fructans and FOS content are presented in **Table 18,** as mean±SD, of the three individual composite samples of these foods.

Table 18. Edible portion, moisture, inulin-type fructans, fructo-oligosaccharide content in potential food sources

Common name	**Edible portion[1] (%)**	**Moisture[1]**	**Inulin-type fructans[1]**	**FOS [1, 2]**
		(g/100g FW)		
Garlic, common	88.48±3.64	65.80±0.70	22.44±2.86	0.94±0.04
Garlic, Chinese	89.89±1.31	69.09±1.36	24.29±1.94	1.74±0.96
Garlic, great headed	91.81±3.77	61.40±0.70	29.16±5.62	1.63±1.42
Jerusalem artichoke	77.47±3.25	73.44±0.26	19.36±1.04	5.18±0.04
Onion, red	91.44±2.57	86.16±0.48	3.56±0.95	3.09±0.54
Shallot	86.55±0.85	83.66±0.92	8.86±0.75	4.98±0.51

(1) Values obtained from analysis of three composite samples from three representative markets

(2) Sum of individual fructo-oligosaccharide: GF_2=1-kestose (1-ketotriose) + GF_3=nystose (1,1-ketotetraose) + GF_4=1F-β-fructofuranosylnystose (1,1,1-ketopentaose)

5.3.1 Inulin-type fructans and fructo-oligosaccharide content

The descending order of the inulin content in the potential foods sources was great headed garlic > Chinese garlic > common garlic > Jerusalem artichoke > shallot > red onion (29.16±5.62, 24.29±1.94, 22.44±2.86, 19.36±1.04, 8.86±0.75 and 3.56±0.95 g/100g FW, respectively).

Content of fructo-oligosaccharide (FOS) in descending order was Jerusalem artichoke > shallot > red onion > Chinese garlic > great headed garlic > common garlic (5.18±0.04, 4.98±0.51, 3.09±0.54, 1.74±0.96, 1.63±1.42 and 0.94±0.04 g/100g FW, respectively).

5.3.2 Sugar content

The results of sugar content are presented in **Table 19,** as mean±SD, of the three individual composite samples of these foods.

Table 19. Sugar content (mean±SD) in potential sources of three individual composite samples

Common name	**Sugar (g/100g FW)**[1]			
	Fructose	**Glucose**	**Sucrose**	**Sum**
Garlic, common	0.13±0.01	0.09±0.02	0.96±0.04	1.18±0.05
Garlic, Chinese	0.20±0.10	0.21±0.21	1.02±0.47	1.43±0.57
Garlic, great headed	0.15±0.04	0.14±0.09	1.14±0.59	1.40±0.55
Jerusalem artichoke	0.34±0.29	0.04±0.02	1.51±0.31	1.89±0.50
Onion, red	0.82±0.27	1.75±0.95	1.33±0.48	3.90±1.66
Shallot	0.35±0.01	0.18±0.01	1.41±0.07	1.94±0.08

[1] Values obtained from analysis of three composite samples from three representative markets

CHAPTER VI
DISCUSSION

Inulin-type fructans and fructo-oligosaccharides (FOS) play important roles in health in many clinical trials. They provide beneficial effects for health and may prevent diseases or promote health as functional foods, giving both dietary fiber and prebiotic effects [72, 73]. Many studies revealed that inulin and FOS were found in a lot of plants as storage carbohydrates in the forms of roots, tubers, vegetables, spices, and fruits, for example, chicory root, Jerusalem artichoke, shallot, garlic, onion and banana [10, 11]. However, there is variation in the quantity depending on plant varieties, degree of maturity and various conditions of cultivation as well as the moisture content in various plants [74]. Data on inulin-type fructans and FOS are not available in the Thai food composition database. The objective of this study was therefore to investigate good sources of inulin among Thai foods. Representative Thai plant foods were obtained from the market place for a preliminary study to identify potential food sources of inulin. Selection of plant foods for the preliminary study was based on the potential food sources reported in previous studies because inulin and FOS are usually stored in organs such as bulbs, tubers and tuberous roots [10, 11, 75]. Another two sets of the identified potential food sources from the preliminary study were sampled again from two more representative markets in Bangkok, Thailand and reanalysed to get a representative database of inulin and FOS.

6.1 Inulin-type fructans and fructo-oligosaccharide content

Inulin-type fructans are a kind of polysaccharides consisting of linear or branching fructose polymers with and without a terminal unit of glucose. Thus, the amount of inulin in the studied samples was determined as the amounts of individual sugars (fructose, glucose and sucrose) after inulinase treatment minus the amounts of the existing sugar in the original sample. A high temperature gas chromatograph was used to detect sugar and quantify the components of fructo-oligosaccharide as 1-kestose (GF_2), nystose (GF_3) and 1F-β-fructofuranosylnystose (GF_4). The main reason for using gas chromatography (GC) for sugar and FOS analyses rather than high pressure liquid chromatography (HPLC) was to obtain good resolution of rhamnose (internal standard), fructose, glucose and sucrose. In addition, the information of individual FOS (degree of polymerization from 3 to 10) was obtained by the GC technique.

In Thai plant foods, potential sources of inulin-type fructans (containing inulin between 19-29 g/100g FW) were found in several varieties of garlic (great headed garlic, Chinese garlic, and common garlic) and Jerusalem artichoke. It was also found that Jerusalem artichoke, red onion and shallot were potential sources of FOS (sum of GF_2, GF_3 and GF_4 = 3-5 g/100g). Potential sources of inulin-type fructans and FOS were found in Jerusalem artichoke, shallot and garlic, which agrees well with previous studies [10, 11].

Only limited data of inulin derived from Van Loo et al., 1995 [10] are available for comparison (**Table 20)**. In the high inulin containing foods, the level of inulin in garlic from this study was higher than the reported values (22.4$\pm$2.9 compared to 9.0-16.0 g/100g FW), whereas that of Jerusalem artichoke was in the same range (16-20 g/100g FW). Among the low inulin containing foods**,** the level in ripe common banana (0.4 g/100g) was at the same range as reported by Van Loo et al. (1995) (0.3 - 0.7 g/100g). On the other hand, lower levels of inulin in leek, common onion, and asparagus were found (0.48, 0.44, 0.02 g/100g compared to the reported data of 3.0-10.0, 1.1-7.5 and 2.0-3.0 g/100g, respectively). Several factors, such as differences in variety, maturity, and moisture content of plant foods could be the responsible factors in the differences [74].

Table 20. Comparison with data from the literature of inulin-type fructans content in Thai foods

Food	Van Loo et al., 1995 (g/100g FW)	This study (g/100g FW)
garlic	9.0-16.0	22.44 ± 2.86 (1)
Jerusalem artichoke	16.0-20.0	19.61 ± 3.74 (2)
banana	0.3-0.7	0.40 (3)
onion, common	1.1-7.5	0.44 (3)
leek	3.0-10.0	0.48 (3)
asparagus	2.0-3.0	0.02 (3)

(1) Values obtained from analysis of three composite samples from three representative markets

(2) Values obtained from duplicate analysis of three composite samples of three varieties of Jerusalem artichoke

(3) Values obtained from duplicate analysis of a single composite sample (each prepared from three representative single samples) collected from one market

For fructo-oligosaccharide (FOS), the available data in the literature were presented on wet weight [10, 75] and on dry weight basis [11] without data on moisture content. For comparison purpose, the FOS data obtained from this study were based on both dry weight and fresh weight (**Table 21)**.

In the high FOS containing foods, the inulin level in Jerusalem artichoke from this study was found lower than those reported by Van Loo et al. (1995), on a wet weight basis (5.18 ± 0.04 compared to 12.0-15.0 g/100g) and by Campbell et al. (1997), on a dry matter basis (19.50 ± 0.17 compared to 28.6 g/100g). On the other hand, the levels of FOS in shallot, red onion and common garlic were much higher than those reported by Campbell et al. (1997), however, Van Loo et al. (1995) reported a higher level in common garlic (on fresh weight basis). Among the low FOS containing foods, the level of FOS in onion, which was determined from a single composite sample from one market, was in accordance with that reported by Campbell et al. (1997), whereas a lower level was found by Van Loo et al. (1995). Compared to the level in leek, data reported by Campbell et al. (1997) were much lower than that found in this study, 0.48 compared to 2.23 g/100g DW. On the other hand, Van Loo et al. (1995) found a much higher level than our finding (2.4-8.0 compared to 0.23 g/100g FW). The levels of FOS in ripe and unripe banana in this study were lower than those reported by Campbell et al. (1997), on a dry matter basis, and by Van Loo et al. (1995) and Hogarth et al. (2000), on a fresh weight basis. For other foods, Van Loo et al. (1995), Campbell et al. (1997) and Hogarth et al. (2000) also reported low levels of FOS. The low level of FOS naturally found in these foods could be one of the factors contributing to the wide range of the reported FOS levels.

6.2 Estimated intake of inulin-type fructans and fructo-oligosaccharide content from selected foods

Estimates of daily inulin consumption from various natural foods ranged from 1 to 4 g for Americans and up to 11 g for Europeans [10]. However, the recommended daily intake (RDI) of inulin- type fructans and FOS has not yet been established. The amount of supplemented inulin in commercial products varies from 2 to 3 g per serving in functional beverages [13].

In Thai foods, the most commonly consumed and possible potential sources of inulin and FOS are starchy roots and tubers, vegetables and legumes, fruits, spices, nuts and seeds. The national food consumption survey data of Thailand in 2002-2004 showed that Thai people aged 19-35 years old consumed foods in these groups at the amount of 10-100 g per eater only per day [76]. The inulin intake of each food was calculated based on the average consumption data (eater only) reported by Kosukwat et al. [76] as presented in ascending order in **Table 22**.

In Thai style cooking, garlic is commonly used as an ingredient in various dishes, for example as a condiment in fried vegetable or meat to increase flavor, or is used as an ingredient of seasoning mixtures for meat before frying. The average amount of garlic consumption for these purposes is about 10 g. Estimated intake of the studied garlic would provide an inulin level of 2.2 to 2.9 g and FOS of 0.1 to 0.5 g/eater only/day, depending on varieties (great headed garlic, Chinese garlic, common garlic). Shallot and red onion are commonly used in various kinds of salad, with average amount of consumption of 10 g and 30 g, respectively. This would provide inulin at 0.9 and 1.1 g and FOS at 0.5 and 0.9 g/eater only/day, respectively.

Table 21. Comparison of fructo-oligosaccharide (FOS) content in Thai foods with data from the literature

Food	Fructo-oligosaccharide (FOS)*				
	Campbell et al., 1997 (g/100g DM)	Van Loo et al., 1995 (g/100g FW)	Hogarth et al., 2000 (g/100g FW)	This study g/100g FW	This study (g/100g DM)
Jerusalem artichoke	28.6	12.0-15.0	-	5.18$\pm$0.04[1]	19.50$\pm$0.17[1]
Shallot	5.29	-	-	4.98$\pm$0.51[2]	29.81$\pm$1.33[2]
Onion, red	1.47	-	-	3.09$\pm$0.54[2]	22.24$\pm$3.21[2]
Garlic, common	1.03	3.6-6.4	-	0.94$\pm$0.04[2]	2.74$\pm$0.07[2]
Onion, white	3.20	1.1-7.5	0.04	0.22[2]	3.03[3]
Leek	0.48	2.4-8.0	-	0.23[2]	2.23[3]
Banana, ripe	1.09	-	-	0.09[2]	0.26[3]
Banana, unripe	0.60	0.3-0.7	0.13	0.02[2]	0.05[3]
Yam	0.09	-	<0.02	0.21[2]	0.25[3]
Potato, sweet	0.08	-	0.03	0.14[2]	0.48[3]
Taro, small, root	0.01	-	-	0.13[2]	0.39[3]
Carrot (Bunny Luv, Dole)	0.14-0.22	-	-	0.04[2]	0.39[3]
Chinese chive	0.11	-	-	0.03[2]	0.24[3]

* Sum of individual FOS: GF_2=1-kestose (1-ketotriose) + GF_3=nystose (1,1-ketotetraose) + GF_4=1F-β-fructofuranosylnystose (1,1,1-ketopentaose)
[1] Values obtained from duplicate analysis of one composite samples from three varieties of Jerusalem artichoke
[2] Values obtained from analysis of three composite samples from three representative markets
[3] Values obtained from duplicate analysis of single samples collected from one market

Table 22. Estimated inulin intake (g/day) of Thai people (eater only) age 19-35 years old

Food Item	Amount consumed/eater only/day (g)(1)	Inulin content (g/100g)(2)	Estimated inulin intake (g/day)
Asparagus	63.20	0.02	0.01
Common Asiatic weed	11.77	0.08	0.01
Lady's finger, Okra	45.10	0.03	0.02
Turmeric, white	87.75	0.02	0.02
Pumpkin	59.52	0.03	0.02
Chinese chive	30.12	0.06	0.02
Bitter cucumber, Chinese	55.54	0.05	0.03
Sweet potato, white	61.74*	0.05	0.03
Horse-tamarind	20.01	0.21	0.04
Yam bean	72.28	0.06	0.05
Banana, common, unripe	69.62	0.06	0.05
Sweet potato, yellow	61.74*	0.13	0.08
Radish	61.07	0.15	0.09
Sunflower seed	30.91	0.34	0.11
Turmeric	87.75	0.13	0.11
Onion	30.15	0.40	0.12
Sweet potato, red	61.74*	0.19	0.12
Leek	30.12	0.48	0.14
Dragon fruits, white	103.34	0.15	0.15
Bitter bean/Sataw	43.40	0.36	0.16
Dragon fruits, red	103.34	0.21	0.21
Banana, rice	63.18	0.34	0.22
Sweet potato	88.94	0.27	0.24
Cassava	67.28	0.36	0.25

Table 22. Estimated inulin intake (g/day) of Thai people (eater only) age 19-35 years old (continued)

Food Item	**Amount consumed/day (g)**$^{(1)}$	**Inulin content (g/100g)**$^{(2)}$	**Estimated inulin intake (g/day)**
Banana, common, ripe	69.62	0.40	0.28
Garden peas	46.61	0.62	0.29
Noh Kala shoot	61.74*	0.52	0.32
Onion, red	30.15*	3.56$^{(3)}$	1.07
Shallot	8.97	8.86$^{(3)}$	0.88
Garlic, Great headed	9.38*	29.16$^{(3)}$	2.74
Garlic	9.38	22.44$^{(3)}$	2.28
Garlic, Chinese	9.38*	24.29$^{(3)}$	2.41
Jerusalem artichoke	72.28*	19.42$^{(4)}$	14.0

(1) Averaged amount of consumption (eater only) from food consumption of Thai people, age 19-35 years [76].
(2) Inulin content in this study (values obtained from duplicate analysis of single composite samples collected from one market)
(3) Values obtained from analysis of three composite samples from three representative markets
(4) Values obtained from duplicate analysis of one composite samples from three varieties of Jerusalem artichoke
* Estimated amount of consumption from the same foods

Jerusalem artichoke can be consumed as fresh salad – one of the common household dishes in Thailand. It can be used as one of the ingredients (replacing potato) in the Indian-type curry, namely Mas-sa-mun, as estimated from the amount consumed per day of yam bean about 70 g. This amount of intake would provide inulin of about 13.6 g and FOS of about 3.6 g/eater only/day, respectively. The actual consumption levels of inulin-type fructans and FOS tended to vary depending upon variations in the component levels that might be due to the differences in plant varieties, degree of maturity and various conditions of cultivation as well as the moisture content in various plants [74].

The daily consumption of inulin at an effective dose may be helpful to stimulate the growth of bifidobacteria in the human colon. Nowadays, in Thai commercial food products, the inulin level of food fortification varies from 2 to 3 g per serving. The intake level of this substance may be lower than the recommended intake of inulin for specific purpose such as for improving mineral absorption, especially calcium, reducing serum cholesterol concentrations and reduction of carcinogens. Numerous human studies have demonstrated these effects starting from 5 g per day. Based on selected foods in this study, only Jerusalem artichoke at the amount consumed by the eaters would provide high amounts of inulin (about 13.6 g). It could be an excellent source of inulin provides prebiotic effect and soluble dietary fiber properties. The usual consumption of high sources of inulin may relieve constipation, decrease the risk of osteoporosis, reduce the risk of atherosclerosis and stimulate the immune system of the body. Thus, the above potential plant foods sources for inulin and FOS reported in this study should be promoted for human consumption, if possible either fresh or as ingredients in the habitual diet. They can also be processed and used as sources of dietary fiber fortificant in commercial products.

At present, growing of Jerusalem artichoke is very limited, only in some areas in the Northeastern part of Thailand; production in other parts of the country should also be encouraged. As variation in inulin level was observed, selection of Jerusalem artichoke varieties with high inulin level should be taken into consideration. Further investigation on this aspect is still required.

CHAPTER VII
CONCLUSIONS

Most studied Thai plants in this study had low amounts of inulin (less than 1 g/100g of fresh weight, FW). However, high amounts of inulin were found in several species. Several varieties of garlic (great headed garlic, Chinese garlic, and common garlic) and Jerusalem artichoke (Kaentawan) contained high amounts of inulin (the levels of 19-29 g/100g FW) followed by shallot and red onion (levels of 3-8 g/100g FW). Jerusalem artichoke, red onion and shallot were also promising sources of fructo-oligosaccharides (FOS) (sum of GF_2, GF_3 and GF_4 = 3-5 g/100g).

Great headed garlic, Chinese garlic, common garlic, Jerusalem artichoke (Kaentawan), shallot and red onion should be promoted for human consumption as fresh or as ingredients in the habitual diet. Estimated intake of any variety of garlic in the study would provide inulin level of 2.2 to 2.9 g and FOS of 0.1 to 0.2 g per eater only per day. Shallot and red onion would provide inulin of 0.9 and 1.1 g and FOS of 0.5 and 0.9 g per eater only per day, respectively. Estimated intake of Jerusalem artichoke per day (70g) would provide an excellent source of inulin (about 13.6 g) and FOS (about 3.6 g). The usual consumption of good sources of inulin may be preventing the risk of many diseases and related to better health and well-being. Hence, they (especially Jerusalem artichoke) can also be processed and used as inulin and FOS sources for prebiotic effect and dietary fiber fortification in the food industry.

REFERENCES

1. Thailand health profile report 2005-2007. Available from: http://www.moph.go.th/ops/thp/index.php?option=com_content&task=view&id=6&Itemid=2&lang=en [Accessed 2009 November 26].
2. Slavin JL. Position of the American dietetic association: health implications of dietary fiber. J Am Diet Assoc 2008; 108(10): 1716-31.
3. Spiller AG. CRC handbook of dietary fiber in human nutrition, Boca Raton, Fla.: CRC Press; 2001.
4. Anderson JW, Baird P, Davis RH Jr, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL. Health benefits of dietary fiber. Nutr Rev 2009; 67(4): 188-205.
5. Roberfroid MB. Caloric value of inulin and oligofructose. J Nutr 1999; 129(7): 1436-7.
6. Roberfroid MB. Dietary fiber, inulin and oligofructose: a review comparing their physiological effects. Crit Rev Food Sci Nutr 1993; 33(2): 103-48.
7. Van den Heuvel EGHM, Muys T, van Dokkum W, Schaafsma G. Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr 1999; 69(3): 544-8.
8. Davidson MH, Maki KC. Effects of dietary inulin on serum lipids. J Nutr 1999; 129(7): 1474S-7S.
9. Gibson GR, Beatty ER, Wang X, Cummings J. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterol 1995; 108: 975–82.
10. Van Loo J, Coussement P, DeLeenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the Western diet. Crit Rev Food Sci Nutr 1995; 35: 525-52.

11. Campbell JM, Bauer LL, Fahey GC, Jr., Hogarth AJCL, Wolf BW, Hunter DE. Selected fructo-oligosaccharide (1-Kestose, Nystose, and 1^F-β-Fructofuranosylnystose) composition of foods and feeds. J Agric Food Chem 1997; 45: 3076-82.
12. ORAFTI. Beneo, nutritional benefits. Available from: http://www.orafti.com [Accessed 2009 November 26].
13. Ray Sahelian MD. Health benefit of inulin supplements. Available from: http://www.raysahelian.com/inulin.html [Accessed 2009 November 26].
14. Fuchs A. Current and potential food and non-food applications of fructans. Biochem Soc Trans 1991; 19: 555–72.
15. Waterhouse AL, Chatterton NJ. Glossary of fructan terms. In: M Suzuki, NJ Chatterton, ed. Science and technology of fructans. CRC Press, Boca Ratan, FL 1993: pp. 2–7.
16. Leenheer LD. Production and use of inulin: Industrial reality with a promising future. In: Prof. Dr. Van Bekkum H, Röper H, Voragen F, ed. Carbohydrates as organic raw materials III 1996: pp. 67-92.
17. DeLeenheer L, Hoebregs H. Progress in the elucidation of the composition of chicory inulin. Starch 1994; 46: 193.
18. Food and Agriculture Organization of the United Nations. Carbohydrates in human nutrition. Vol. 66. Rome, Report of a joint FAO/WHO Expert Consultation 1998.
19. Wikipedia, the free encyclopedia.mht. Fructan. Available from: http://en.wikipedia.org/wiki/Fructan [Accessed 2009 November 26].
20. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr 1995; 125(6): 1401-12.
21. Kono T. Fructooligosaccharides. In: Nakakuki T, ed. Oligosaccharides: production, properties, and applications. Vol. 3. Sydney, Australia: Gordon and Brech Sciences Publishers 1993: pp.50-78.
22. Praznik W, Beck RHF. Inulin composition during growth of tubers of *Helianthus tuberosus* L. Agric Biol Chem 1987; 51: 1593–9.

23. Kocsis L, Liebhard P, Praznik W. Effect of seasonal changes on content and profile of soluble carbohydrates in tubers of different varieties of Jerusalem Artichoke (*Helianthus tuberosus* L.). J Agric Food Chem 2007; 55: 9401–8.

24. Vorasoot N, Jogloy S. Inulin: Non-digestible carbohydrate as soluble fiber from Kaentawan for human health. Khon Kaen Agric 2006; 34(2): 85-91.

25. Roberfroid MB, Gibson GR, Delzenne N. The biochemistry of oligofructose, a nondegestible fiber: An approach to calculate its caloric value. Nutr Rev 1993; 51: 137–46.

26. American Association of Cereal Chemists. The definition of dietary fiber. Cereal Foods World 2001; 46(3): 112-27.

27. Knudsen KEB, Hessov I. Recovery of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) in the small intestine of man. Bri J Nutr 1995; 74: 101-13.

28. Delzenne N, Roberfroid MB. Physiological effects of nondigestible oligosaccharides. Lebensm Wiss Technol 1994; 27: 1-6.

29. Roberfroid M, Van Loo J, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr 1998; 128: 11–9.

30. Jackson KG, Taylor GRJ, Clohessy AM, Williams CM. The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. Br J Nutr 1999; 82: 23–30.

31. Leeds AR. Glycemic index and heart disease. Am J Clin Nutr 2002; 76: S286-9.

32. Yamashita K, Kawai K, Itakura M. Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutr Res 1984; 4: 961-6.

33. Brighenti F, Casiraghi M, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur J Clin Nutr 1999; 53: 726–33.

34. Canzi. E., Brighenti F, Casiraghi MC, Del Puppo E, Ferrari A. Prolonged consumption of inulin in ready to eat breakfast cereals: effects on intestinal ecosystem, bowel habits and lipid metabolism. In: COST 92 Workshop. Dietary Fibre and Fermentation in the Colon. Luxembourg: Office for Official Publications of Tile European Communities, 1992: 280–4.

35. Hond ED, Geypens B, Ghoos Y. Effect of high performance chicory inulin on constipation. Nutr Res 2000; 20: 731–6.
36. Kleessen B, Svkura B, Zunft H-J, Blaut M. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am J Clin Nutr 1997; 65: 1397-402.
37. Wong JM, de Souza R, Kendall CW, Emam A, DJJ. Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol 2006; 40 (3): 235–43.
38. Cashman K. Calcium intake, calcium bioavailability and bone health. Br J Nutr 2002; 87(Suppl 2): S169–77.
39. Coudray C, Bellanger J, Castı´glia-Delavaud C, Vermorel V, Rayssignuier Y. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 1997; 51: 375–80.
40. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr 2005; 82(2): 471-6.
41. Holloway L, Moyniham S, Abrams SA, Kent K, Hsu AR, Friedlander AL. Effects of oligofructose-enriched inulin on intestinal absorption of calcium and magnesium and bone turnover markers in post-menopausal women. Br J Nutr 2007; 97: 365–72.
42. Williams CM. Effects of inulin on lipid parameters in humans. J Nutr 1999; 129(7): 1471-3.
43. Balcazar-Munoz BR, Martinez-Abundis E, Gonzalez-Ortiz M. Effect of oral inulin fiber administration on lipid profile and insulin sensitivity in subjects with obesity and dyslipidemia. Rev Med Chil 2003; 131(6): 597-604.
44. Letexier D, Diraison F, Beylot M. Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. Am J Clin Nutr 2003; 77(3): 559-64.
45. Pool-Zobel BL. Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. Br J Nutr 2005; 93(Suppl 1): S73–90.

46. Klinder A, Gietl E, Hughes R, Jonkers N, Pistoli S, Gibson G. Gut fermentation products of inulin-derived prebiotics inhibit markers of tumour progression in human colon tumor cells. Int J Cancer Prev 2004b; 1: 19–32.

47. Boutron-Ruault MC, Marteau P, Lavergne-Slove A, Myara A, Gerhardt MF, Franchisseur C. Effects of a 3-mo consumption of short-chain fructo-oligosaccharides on parameters of colorectal carcinogenesis in patients with or without small or large colorectal adenomas. Nutr Cancer 2005; 53: 160–8.

48. Menne E, Guggenbuhl N. Fn-type chicory inulin hydrolysate has a prebiotic effect in humans. J Nutr 2000; 130(5): 1197-9.

49. Wang X, Gibson GR. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacteriol 1993; 75: 373–80.

50. Roberfroid MB. Prebiotics: preferential substrates for specific germs?. Am J Clin Nutr 2001; 73(suppl): 406S–9S.

51. Elzbieta B, Maria B. Prebiotic effectiveness of fructans of different degrees of polymerization. Trends Food Sci Tech 2004; 15: 170-5.

52. Rao VA. The prebiotic properties of oligofructose at low intake levels. Nutr Res 2001; 21(6): 843-8.

53. Krusse H, Kleessen B, Blaut M. Effect of inulin on faecal bifidobacteria in human subjects. Br J Nutr 1999; 82 (5): 375-82.

54. Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify the mucosa associated microflora of the human large bowel. Gut 53. 2004: 1610–6.

55. Stephanie. S, Berhard W. Inulin and Oligofructose: Review of experimental data on immune modulation. J Nutr 2007; 137: 2563-67S.

56. Anne-Judith W-D, Florence C, Loannis N, Anne B, Pascale S, Christophe D, Marie-Jose B. Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. Int J Food Microbiol 2007; 113(1): 108-13.

57. U.S. Department of Agriculture Agricultural Research Service. 1994-1996 Continuing Survey of Food Intakes by Individuals and 1994-96 Diet and Health Knowledge Survey. In: National Technical Information Service, ed. Springfield, Va. 1998.

58. Coussement P. Inulin and oligofructose: Safe intakes and legal status. J Nutr 1999; 129: 1412S–7S.

59. Mead Johnson Nutrition. Product. Available from: http://www.enfababy.com/2009/product/detail/3/ [Accessed 2010 January 25].

60. Tungland BC. Inulin - A comprehensive scientific review 2000. Available from: http://members.shaw.ca/duncancrow/inulin_review.html [Accessed 2010 January 25].

61. Absolonne J, Jossart M, Coussement P, Roberfroid M. Digestive acceptability of oligofructose. Proc. lst Iraqi Research Conf 1995; 135-144.

62. Takahashi Y, Takagi Y, Toda M, Kashiwabara C, Ishiyama R, Kinoshita T. Effects of neosugar in chronic renal-failure patients. In: Proc, third ed., Tokyo, Japan, 110. Neosugar Research Conf 1986.

63. Spiegel JE, Rose R, Karabell P, Frankos VH, Schmitt FD. Safety and benefits of fructooligosaccharides as food ingredients. Food Technol 1994; 48: 85-9.

64. Issariyachaikul K. Development of modified fat ice cream products using inulin as a fat replacer, (M.S. Thesis in Food and Nutrition for Development). Bangkok: Faculty of Graduate Studies, Mahidol University; 2008.

65. Sukheesab K. Formulation of wheat flour and inulin mix for bread made by different methods, (M.S. Thesis in Food and Nutrition for Development). Bangkok: Faculty of Graduate Studies, Mahidol University; 2007.

66. Quemener B, Thibault JF, Coussement P. Determination of inulin and oligofructose in food products, and integration in the AOAC method for measurement of total dietary fibre. Lebensm Wiss Technol 1994; 27: 125–32.

67. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. In: Horwitz W. editor, 18th ed., Methods: 997.08, 985.29, 990.19. Maryland. AOAC Int. 2005.

68. Hoebregs H. Fructans in foods and food products. Ion-exchange chromatographic method: Collaborative study. J AOAC Int. 1997; 80: 1029−37.
69. McCleary BV, Murphy A, Mugford DC. Determination of oligofructans and fructan polysaccharides in foodstuffs by an enzymatic/spectrophotometric method: collaborative study. J AOAC Int. 1997; 83: 356-64.
70. Vendrell-Pascuas S, Castellote-Bargalló AI, López-Sabater MC. Determination of inulin in meat products by high-performance liquid chromatography with refractive index detection. J Chromatogr A 2000; 881: 591−7.
71. Joye D, Hoebregs H. Determination of oligofructose, a soluble dietary fiber, by high-temperature capillary gas chromatography. J AOAC Int. 2000; 83: 1020-5.
72. Roberfroid MB, Delzenne NM. Dietary fructans. Annu Rev Nutr 1998; 18: 117–43.
73. Roberfroid MB. Inulin-type fructans: functional food ingredients. J Nutr 2007; 137: 2493S–502S.
74. Greenfield H, Southgate DAT. Food composition data: production, management and use. In: Burlingame B.A. and Charrondiere U.R. editor, 2nd ed., Food and Agriculture Organization of the United Nations. Rome, 2003.
75. Hogarth AJCL, Hunter DE, Jacobs WA, Garleb KA, Wolf BW. Ion chromatographic determination of three fructooligosaccharide oligomers in prepared and preserved foods. J Agric Food Chem 2000; 48: 5326-30.
76. Kosukwat V, Rojroongwasinkul N, Boonpraderm A, Viriyapanich T, Jitnarin N, Sornkaew N, Wanijjakul C. Food consumption of Thai population. Institute of Nutrition, Mahidol University, Bangkok Thailand, 2006.

APPENDICES

APPENDIX A

The illustration of raw Jerusalem Artichoke or Kaentawan

(Helianthus Tuberosus L.*)*

APPENDIX B

Determination of moisture
Hot air oven method, based on AOAC 2005, 990.19

Principle:

A well homogeneous sample is dried in an oven (usually at 100±5 ºC) unit constant weight is obtained. The loss of weight is taken as a measure of moisture content in the sample. Acid washed sand is used to mix with the wet sample prior to dry in order to increase area for rapid and complete evaporator of water from the wet sample.

Procedure:

1. Weigh approximately 20 g of acid washed sand into a porcelain dish containing a small glass stirring rod and dry in hot air oven at 100±5 ºC for 30 min.
2. Remove the sand dish and cool in the desiccator.
3. Weigh sand dish (=*a* g) and then approximately 5 g sample. Reweigh (=*b* g).
4. Add small amount of deionised water to disperse the sample evenly and evaporate the water as mush possible on the boiling water bath. The sample dish should be frequently mixed until dry.
5. Transfer the sample dish to hot air oven and dry the sample at 100±5 ºC for 2 hrs.
6. Remove the sample dish and cool in desiccator and weigh (=*c* g).
7. Return the sample dish to the hot air oven and dry unit a constant weigh is obtained. Reweigh every 30 min.
8. The different weigh between each interval time should not be more than 1-3 mg.

Calculation:

$$\% \text{ Moisture} = \frac{(b-c) \times 100}{(b-a)}$$

where a = weight of sand and dish

b = weight of sand, dish and sample

c = weight of sand, dish and sample after drying and cooling to room temperature

APPENDIX C

Determination of fructans, based on AOAC 2005, 997.08

Principle:

Fructans are extracted from the product with boiling water. Aliquot of extract is hydrolyzed using lyophilized amyloglucosidase to remove starch. Part of that hydrosylate is treated with inulinase followed by determination of released sugars.

Procedure:

1. Weigh dried sample approximately 1 to 5 g (~1 g of fructan) into a 100 mL screwed cap bottle and extract with hot water (~50 mL).
2. Adjust pH of solution to 6.5-8.0 with 0.05 N KOH or 0.05 N HCl , make up to 50 mL with deionised water and incubate at 85±2ºC in shaking water-bath for 15 min.
3. Weigh extracted sample solution (~5 g) into a 25 mL volumetric flask (contained 0.01 g of rhamnose as an internal standard) and dilute with deionised water (25 mL).
4. Weigh extract sample solution (~15 g) into a 100 mL screwed cap bottle (for hydrolysis with enzyme), add acetate buffer (~15 g), adjust pH to 4.5±0.5 with 0.2 M acetic acid or 0.2 M Na-acetate and add sufficient amount of inulinase and incubate for 30 min in shaking water bath, at 60±2 ºC.
5. Weigh solution after hydrolysis (~5 g) into a 25 mL volumetric flask (contained 0.01 g of rhamnose) and dilute with deionised water (25 mL).
6. Pipette (200 µL) solution of extracted samples and solution after hydrolysis (from items 3 and 5) into 10 mL screwed-cap tubes and dry in a vacuum desiccator until dryness (~ 3 h).
7. Add 400 µL of hydroxylamine chloride in pyridine into each tube, mix the solution for 10 min on vortex mixer; incubate in a water bath at 60 ºC for 5 min and centrifuge at 2000 rpm for 5 min.

8. Add 200 µL of silylating agent (tri-methysilylimidazole, TSIM) into each tube and shake for 10 min on vortex mixer.
9. Add 1000 µL of deionised water, 2000 µL of isooctane, shake for 3 min and centrifuge at 2000 x g for 5 min.
10. Transfer upper solution into a gas chromatography (GC) vial and determine sugar content by GC.

Condition of Gas Chromatography (GC):

1. High temperature GC (Agilent® 7890, USA)
    - Column: Al-clad capillary column, 6 m long x 0.53 mm ID, coated with 5%-phenyl-polycarboranesiloxane, HT-5, Restek®, USA
    - Inlet system: cool-on column
    - Detector: Flame Ionization Detector (FID)
      Set temperature at 447 ºC
    - Helium as a carrier gas at 9 ml/min
    - Nitrogen as a make up gas, set at flow rate of 25 mL/min
    - Air and hydrogen gas, set at 400 mL/min and 40 mL/min, respectively.
2. GC (Agilent® 6850, USA)
    - Column: HP-1 (100% dimethylpolysiloxane, Agilent® J&W, USA) capillary fused silica column, length 30 m, ID 0.32 mm.
    - Inlet system: cool-on column
    - Detector: Flame Ionization Detector (FID)
      Set temperature at 350 ºC
    - Helium as a carrier gas at 2 ml/min
    - Nitrogen as a make up gas, set at flow rate of 30 mL/min
    - Air and hydrogen gas, set at 350 mL/min and 35 mL/min, respectively.

Calculation:

1) Calculate concentration of each sugar in sample as follows;

$$C = R \cdot M_0 \cdot \frac{A}{A_0} \cdot \frac{100}{W} \quad \ldots\ldots\ldots \ (1)$$

where; C is concentration of each sugar (mg/100g sample)

R is response factor of each sugar from standard

M_0 is amount of rhamnose added (internal standard) (mg/50 ml)

A_0 is a peak area of rhamnose in sample

A is a peak area of each sugar in sample

W is amount of sample (g/50 ml)

2) Calculate response factor (R) of each sugar standard as follows;

$$R = \frac{M}{M_0} \cdot \frac{A_0}{As} \quad \ldots\ldots\ldots\ldots \ (2)$$

where; R is response factor of each sugar from each standard

M_0 is amount of rhamnose (mg/50 ml)

A_0 is a peak area of rhamnose

M is amount of each standard sugar (mg/50 ml)

As is a peak area of each standard sugar

3) Calculate actual amount of fructose (F_{IN}) derived from inulin after inulinase hydrolysis as follows;

$$F_{IN} = \left[F_{NH} - F_{T_0} - \frac{S_{T_0}}{1.9} \right] \cdot f \quad \ldots\ldots\ldots\ldots\ldots\ldots \ (3)$$

where; F_{IN} is the actual amount of fructose (g/100g) derived from inulin

F_{NH} is the amount of existing fructose (g/100g) in the sample plus fructose which derived from inulin after hydrolysis with inulinase

F_{To} is the amount of existing fructose (g/100g) without enzyme hydrolysis

S_{To} is the amount of existing sucrose (g/100g) without enzyme hydrolysis

f is the factor of DP of standard inulin (>99%) given by Orafti® ST from equation (4) as follow:

$$f = \frac{DS}{INUL + F_{T_0} + G_{T_0} + S_{T_0}} \quad \ldots\ldots\ldots\ldots \quad (4)$$

where; DS is amount of inulin in a standard Raftilose® P95 (g/100g)

$INUL$ is amount of inulin in sample (g/100g)

F_{T0} is amount of fructose before hydrolysis with inulinase enzyme (g/100g)

G_{T0} is amount of glucose before hydrolysis with inulinase enzyme (g/100g)

S_{T0} is amount of sucrose before hydrolysis with inulinase enzyme (g/100g)

5) Calculate actual amount of glucose (G_{IN}) derived from inulin after inulinase hydrolysis as follows;

$$G_{IN} = \frac{F_{IN}}{DP - 1} \quad \ldots\ldots\ldots\ldots\ldots. \ (5)$$

where; G_{IN} is the actual amount of glucose derived from inulin after inulinase hydrolysis

F_{IN} is the actual amount of fructose (g/100g) derived from inulin

DP is an estimated DP of inulin in the unknown sample (100% inulin), calculated by equation (6)

6) Calculate DP of unknown sample as follows;

$$DP = \frac{(Oligo \cdot DP_{Oligo}) + (Inulin \cdot DP_{Inulin})}{Sum \ (Oligo + Inulin)} \quad \ldots\ldots\ldots\ldots. \ (6)$$

7) Calculate amount of inulin-type fructans as follows;

$$\text{Inulin-type fructans (\%)} = (F_{IN}+G_{IN})\times \boldsymbol{f}_{\mathbf{DP}} \quad \ldots\ldots\ldots.. \quad (7)$$

where; F_{IN} is the actual amount of fructose derived from inulin after inulinase hydrolysis, calculated by equation (4)

G_{IN} is the actual amount of glucose derived from inulin after inulinase hydrolysis, calculated by equation (5)

f_{DP} is factor of an estimated degree of polymerisation (DP) of inulin in the unknown sample, derived from equation (8)

$$\boldsymbol{f}_{\boldsymbol{DP}} = \frac{\left[(\boldsymbol{DP}\cdot 180)-((\boldsymbol{DP}-1)\cdot 18)\right]}{(\boldsymbol{DP}\cdot 180)} \quad \ldots\ldots\ldots\ldots \quad (8)$$

APPENDIX D

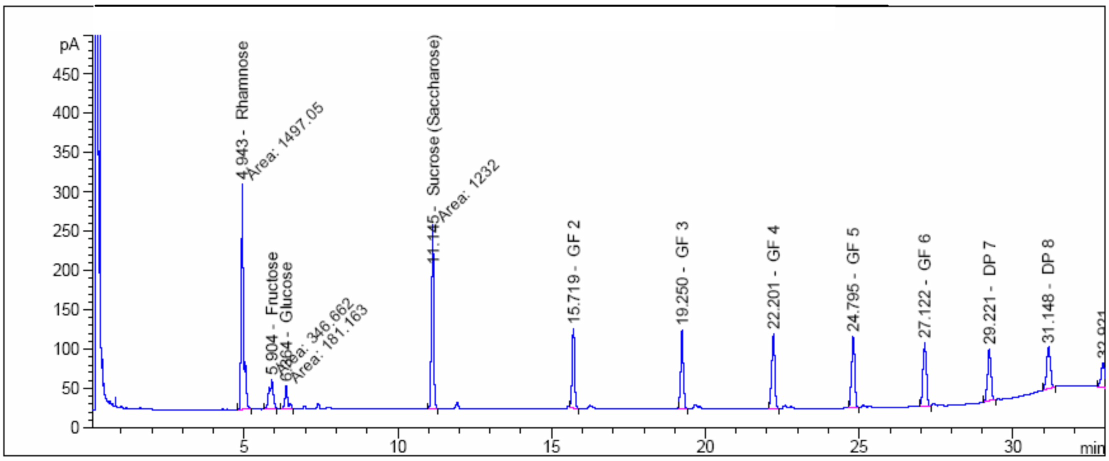

Figure D1 The chromatograms of sugars (fructose, glucose and sucrose) and fructo-oligosaccharide (FOS) (GF_2, GF_3 and GF_4) in extracted sample solution of Beneo (Orafti®, Belgium) (as commercial mixture of inulin**)** from Agilent® 7890 high temperature GC **-** without hydrolysis

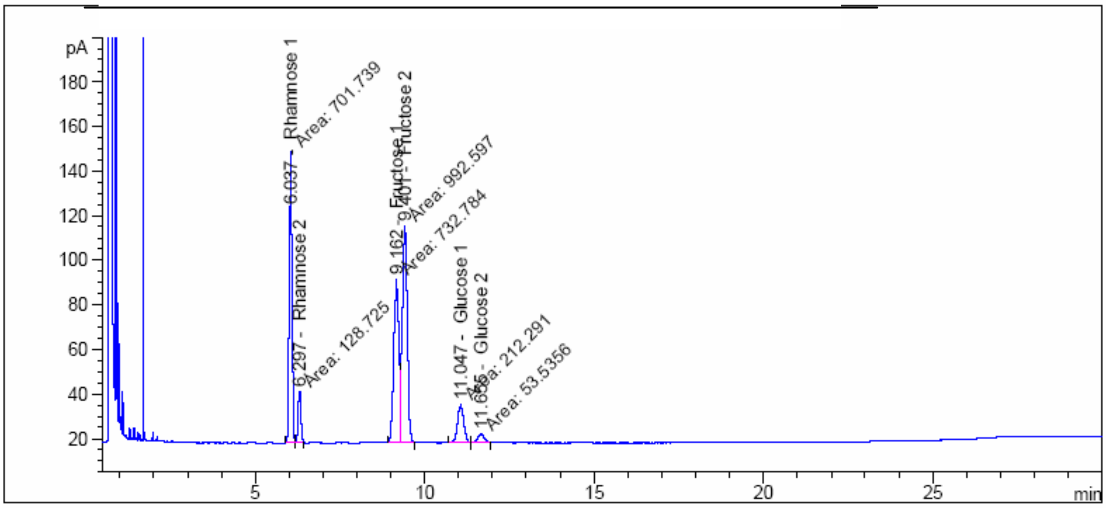

Figure D2 The chromatograms of sugars (fructose, glucose and sucrose) and fructo-oligosaccharide (FOS) (GF_2, GF_3 and GF_4) in extracted sample solution of Beneo (Orafti®, Belgium) (as commercial mixture of inulin**)** from Agilent® 6850 GC – after hydrolysis with enzyme

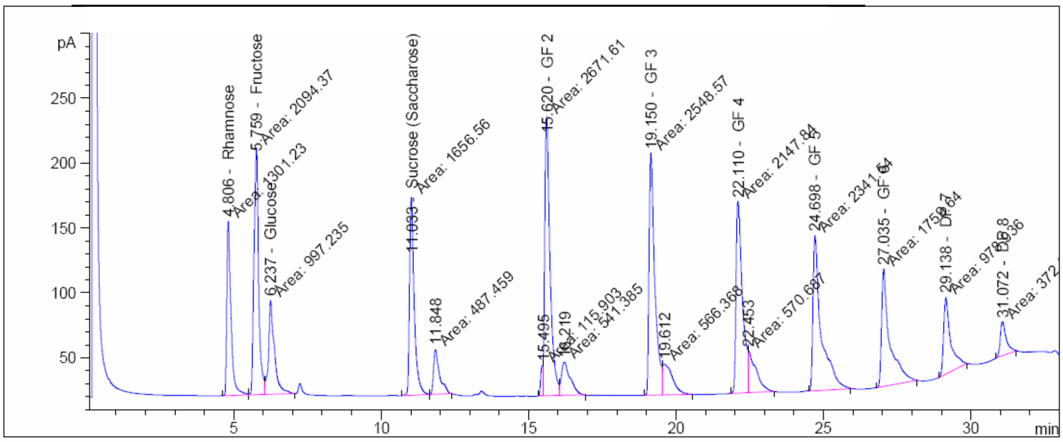

Figure D3 The chromatograms of sugars (fructose, glucose and sucrose) and fructo-oligosaccharide (FOS) (GF_2, GF_3 and GF_4) in extracted sample solution of Jerusalem artichoke (HEL66 variety) using Agilent® 7890 high temperature GC - without hydrolysis

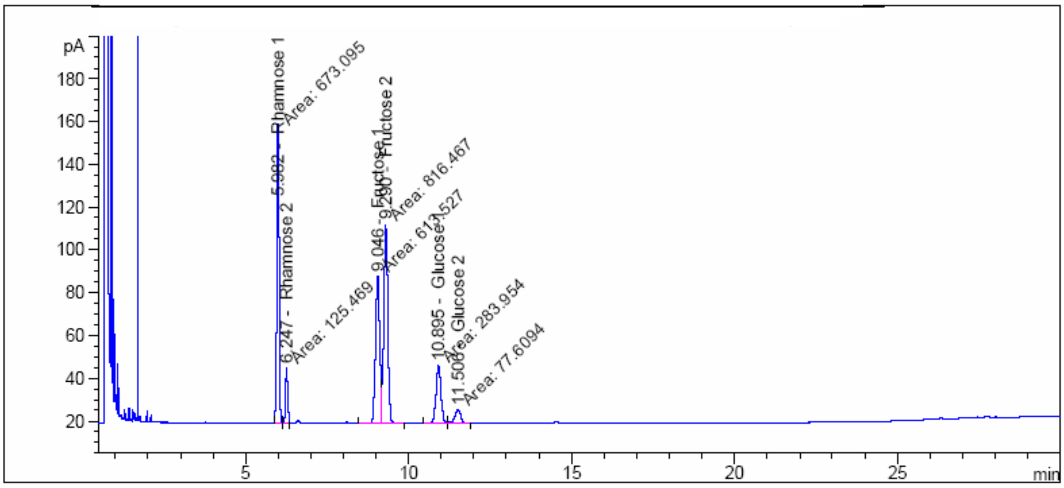

Figure D4 The chromatograms of sugars (fructose, glucose and sucrose) and fructo-oligosaccharide (FOS) (GF_2, GF_3 and GF_4) in extracted sample solution of Jerusalem artichoke (HEL66 variety) using Agilent® 6850 GC – after hydrolysis with enzyme

BIOGRAPHY

NAME	Miss Siriporn Tanjor
DATE OF BIRTH	23 May 1983
PLACE OF BIRTH	Samutsongkram, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 2002-2005
	Bachelor of Science (Nutrition)
	Mahidol University, 2007-2010
	Master of Science (Nutrition)
RESEARCH GRANT	This thesis is partially supported by
	Graduate Studies of Mahidol University
	Alumni Association.
HOME ADDRESS	181/2 Soi Phahonyothin 35
	Phahonyothin Rd., Ladyao,
	Jatujuk, Bangkok. 10900
	Thailand
	Tel: +66 (0) 86-908-9480
	E- mail: s.tanjor@hotmail.com
	tanjor_109@yahoo.com
PUBLICATION / PRESENTATION	Poster presentation at the 8th International
	Food Data Conference (IFDC)
	October 1-3, 2009.
	Bangkok, Thailand
	Poster presentation at the 19th International
	Congress of Nutrition (ICN).
	October 4-9, 2009.
	Bangkok, Thailand