ปริมาณเหล็ก วิตามินซี ฟัยเตท และเส้นใยอาหาร ในผักพื้นบ้านภาคตะวันออกเฉียงเหนือ

นางสาวพนารัตน์ เลาหบุตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาอาหารเคมีและโภชนาศาสตร์ทางการแพทย์ ภาควิชาอาหารเคมี
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IRON, VITAMIN C, PHYTATE AND CRUDE FIBER CONTENTS IN NORTHEASTERN LOCAL VEGETABLES

Miss Panarat Laohabutr

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By	Miss Panarat Laohabutr
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Thesis Advisor	Associate Professor Oranong Kangsadalampai, Ph.D.
Thesis Co-advisor	Associate Professor Thitirat Panmaung, M.Sc.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

……Dean of Faculty of Pharmaceutical Sciences
(Associate Professor Sunibhond Pummangura, Ph.D.)

THESIS COMMITTEE

……Chairman
(Associate Professor Kaew Kangsadalampai, Ph.D.)

……Thesis Advisor
(Associate Professor Oranong Kangsadalampai, Ph.D.)

……Thesis Co-advisor
(Associate Professor Thitirat Panmaung, M.Sc.)

……Member
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

……Member
(Linna Thonyonk, M.Sc. in Pharmacy)

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เหล็กในอาหารแบ่งออกได้เป็น 2 รูปแบบ คือเหล็กที่อยู่ในรูปของฮีม พบในเนื้อสัตว์ ซึ่งร่างกายสามารถดูดซึมได้ดี และเหล็กที่ไม่ได้อยู่ในรูปของฮีม พบในธัญพืช ผัก ผลไม้ ร่างกายดูดซึมได้ไม่ดี การดูดซึมเหล็กที่ไม่ได้อยู่ในรูปของฮีมขึ้นกับสารอื่นในอาหาร ได้แก่ วิตามินซี ช่วยเพิ่มการดูดซึมเหล็ก ส่วนฟัยเตทและเส้นใยอาหาร มีผลยับยั้งการดูดซึมเหล็ก การวิจัยนี้มีจุดมุ่งหมายเพื่อวิเคราะห์หาปริมาณเหล็ก วิตามินซี ฟัยเตท เส้นใยอาหาร และชีวปริมาณออกฤทธิ์ของเหล็กในผักพื้นบ้านภาคตะวันออกเฉียงเหนือ ผลการศึกษาพบว่า ผักแว่นมีปริมาณเหล็กสูงที่สุด ในขณะที่คูนมีปริมาณเหล็กต่ำที่สุด (62.49 และ 0.93 มิลลิกรัมต่อ 100 กรัมน้ำหนักแห้ง ตามลำดับ) ใบย่านางมีปริมาณวิตามินซีสูงที่สุด ในขณะที่ผักผำมีปริมาณวิตามินซีต่ำที่สุด (164.68 และ 8.69 มิลลิกรัมต่อ 100 กรัมน้ำหนักสด ตามลำดับ) สำหรับฟัยเตทพบปริมาณสูงสุดในยอดแค และพบปริมาณต่ำสุดในใบย่านาง (1184.56 และ 32.66 มิลลิกรัมต่อ 100 กรัมน้ำหนักแห้ง ตามลำดับ) ส่วนเส้นใยอาหารพบปริมาณสูงสุดในใบย่านาง และพบปริมาณต่ำสุดในผักผำ (6.56 และ 0.48 กรัมต่อ 100 กรัม น้ำหนักแห้ง ตามลำดับ) และเมื่อศึกษาความสัมพันธ์ระหว่างปริมาณเหล็ก วิตามินซี ฟัยเตท และเส้นใยอาหาร พบว่าปริมาณวิตามินซีมีความสัมพันธ์กับปริมาณเส้นใยอาหารในทางบวก ที่ระดับนัยสำคัญทางสถิติ 0.05 แต่ปริมาณเหล็กมีความสัมพันธ์กับปริมาณฟัยเตทในทางลบ ที่ระดับนัยสำคัญทางสถิติ 0.01

ผลการศึกษาถึงชีวปริมาณออกฤทธิ์ของเหล็กโดยการคำนวณตามวิธีของ Monsen และ Tseng พบว่า ผักแว่นมีปริมาณชีวปริมาณออกฤทธิ์ของเหล็กสูงที่สุด (3.05 และ 1.13 มิลลิกรัมต่อ 100 กรัม โดยวิธีของ Monsen และ Tseng ตามลำดับ) ในขณะที่คูนมีปริมาณชีวปริมาณออกฤทธิ์ของเหล็กต่ำที่สุด (0.04 และ 0.0076 มิลลิกรัมต่อ 100 กรัม โดยวิธีของ Monsen และ Tseng ตามลำดับ) และพบว่าค่าเฉลี่ยของปริมาณชีวปริมาณออกฤทธิ์ของเหล็กตามวิธีของ Monsen เท่ากับ 0.73 ± 0.68 มิลลิกรัมต่อ 100 กรัม สูงกว่าวิธีของ Tseng ซึ่งมีค่าเฉลี่ยเท่ากับ 0.24 ± 0.26 มิลลิกรัมต่อ 100 กรัม ทั้งนี้เนื่องจากวิธีของ Monsen จะพิจารณาเฉพาะปัจจัยที่ช่วยเพิ่มการดูดซึมเหล็กเพียงอย่างเดียว ส่วนวิธีของ Tseng จะพิจารณาทั้งปัจจัยที่ช่วยเพิ่มและยับยั้งการดูดซึมเหล็ก ผลการศึกษานี้จะเป็นข้อมูลพื้นฐานในการศึกษาทางด้านโภชนาการ และการเลือกรับประทานอาหารให้ได้รับเหล็กเพียงพอกับความต้องการของร่างกาย

ภาควิชา.....อาหารเคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา.อาหารเคมีและโภชนศาสตร์ทางการแพทย์. ลายมือชื่ออาจารย์ที่ปรึกษา.....

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4276577733 : MAJOR FOOD CHEMISTRY
KEY WORD: IRON / VITAMIN C / PHYTATE / CRUDE FIBER / VEGETABLE / IRON BIOAVAILABILITY
PANARAT LAOHABUTR: IRON, VITAMIN C, PHYTATE AND CRUDE FIBER CONTENTS IN NORTHEASTERN LOCAL VEGETABLES. THESIS ADVISOR: ASSO. PROF. ORANONG KANGSADALAMPAI, Ph.D. THESIS COADVISOR: ASSO. PROF. THITIRAT PANMAUNG, M.Sc. (FOOD TECH.) 102 pp. ISBN 974-13-0759-4.

Food iron is existed in two forms, heme iron is found in meat, fish and poultry; nonheme iron is found in cereal, vegetable and fruit. The absorption of heme iron is higher than that of nonheme iron. The absorption of nonheme iron can be enhanced or inhibited by various dietary components. The major enhancer of nonheme is vitamin C; the major inhibitors are phytate and fiber. This study determined iron, vitamin C, phytate, crude fiber and iron bioavailability contents in northeastern local vegetables. Iron, the highest was found in *Marsilea crenata* Presl (62.49 mg/100g dry weight) while the lowest was found in *Colocasia gigantea* Hook. f. (0.93 mg/100g dry weight). Vitamin C, the highest was found in *Tiliacora triandra* Diels (164.68 mg/100g wet weight) while the lowest was found in *Wolffia globosa* Hartog & Plas (8.69 mg/100g wet weight). For phytate, the highest was found in *Sesbania grandiflora* (L.) Pers (1184.56 mg/100g dry weight) while the lowest was found in *Tiliacora triandra* Diels (32.66 mg/100g dry weight). Crude fiber, the highest was found in *Tiliacora triandra* Diels (6.56 g/100g dry weight) while the lowest was found in *Wolffia globosa* Hartog & Plas (0.48 g/100g dry weight). The positive significant correlation was found between vitamin C and crude fiber ($P < 0.05$). In contrast, the negative significant correlation was found between iron and phytate ($P < 0.01$).

Iron bioavailability, the highest was found in *Marsilea crenata* Presl (3.05 and 1.13 mg/100g by Monsen's and Tseng's method, respectively) while the lowest was found in *Colocasia gigantea* Hook. f. (0.04 and 0.0076 mg/100g by Monsen's and Tseng's method, respectively). The mean of iron bioavailability from the Monsen's and Tseng's method was 0.73 ± 0.68 mg/100g and 0.24 ± 0.26 mg/100g, respectively. The calculated iron bioavailability by Monsen's method was higher than that by Tseng's method because Monsen' s method was considered only the effect of enhancing factor; while Tseng's method was involved both the effects of enhancing and inhibiting factor. The result of these studies would be meaningful for being a background data of studying in nutritional aspect and selecting consumption in order to meet adequate dietary iron.

Department…..Food Chemistry…… Student's signature…….
Field of Study.Food Chemistry and Medical Nutrition. Advisor's signature…….
Academic year…..2000……. Co-advisor's signature……

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LIST OF ABBREVIATIONS

A.R.	=	Analytical Reagent
°C	=	degree celcius
e.g.	=	exampli gratia (for example)
et al.	=	et alli (and others)
g	=	gram
g/dl	=	gram per decilitre
i.e.	=	id est (that is)
L/min	=	Litre per minute
mA	=	milliampere
mg	=	milligram (s)
mg/d	=	milligram per day
mg/g	=	milligram per gram
mg/kg/d	=	milligram per kilogram per day
ml	=	millilitre (s)
mo	=	month (s)
nm	=	nanometer (s)
ppm	=	part per million
vs	=	versus
yr	=	year
μg	=	microgram
μg/l	=	microgram per liter
μg/ml	=	microgram per millilitre
μmol/kg	=	micromole per kilogram

%	=	percentage
<	=	less than
≥	=	greater than or equal

CHAPTER I

INTRODUCTION

Anemia is known to be one of the major health problems especially in many developing countries and Thailand is no exception. It is particularly prevalent among infants, young children, pregnant and lactating women (Valyasevi, Benchakarn and Dhanamitta, 1974; Stephenson, 1995). One of an important causations was the low availability of dietary iron consumption resulting in iron deficiency (Narasinga Rao and Prabhavathi, 1978). There are two major reasons why the iron in such diets was poorly absorbed. Firstly, many of the foods that were consumed contain powerful inhibitors of iron absorption, namely phytate and crude fiber. Secondly, the diets were usually deficient in the two major promoters of nonheme iron absorption, namely meat and ascorbic acid. Therefore, in evaluating causes of iron deficiency, it was important to consider the availability of dietary iron (Tuntawiroon *et al.*, 1991; Schricker *et al.*, 1981; Rossander, Hallberg and Bjorn-Rasmussen, 1979; Cook, Dassenko and Lynch, 1991).

Several attempts have been made for determining iron availability by various methods. One method which was proved to be very successful was human iron absorption study using the extrinsic radioiron tag method (Layrisse, Martinez-Torres and Gonzalez, 1974; Hallberg *et al.*, 1974). However, this method was so difficult, expensive and time consuming. Even worse, the administration of radionuclides to human was not guaranteed from harm.

Monsen *et al.* (1978) evaluated the results of numerous studies of iron absorption in human subjects and made recommendations for planning and evaluating iron intakes on the basis of two key enhancers of nonheme iron absorption: ascorbic acid and animal tissue. They have proposed a method for calculating absorbable iron. In brief, the amounts of heme and nonheme iron in any particular meal were considered separately because of their different availability and susceptibility to influences from other dietary ingredients.

Recently, Tseng *et al.* (1997) evaluated the effects of enhancing and inhibiting factors on calculated iron absorption. They suggested estimating nonheme iron bioavailability by two steps: first, calculate iron bioavailability using Monsen's method (Monsen *et al.*, 1978; Monsen and Balintfy, 1982); second, adjust the bioavailability of nonheme iron for phytate (Hallberg, Brune and Rossander, 1989).

The fact that the high vegetable diet was consumed by northeastern inhabitants of Thailand. To know the kind of high absorbable iron vegetables will be helpful for the proper selection in order to decrease the risk of iron deficiency. Thus, this study was performed to determine the bioavailability of iron in northeastern local vegetables by calculating method and the correlation between the amount of iron with vitamin C, phytate and crude fiber in northeastern local vegetables.

The objectives of the study

The aims of this study were as follows:

1. To determine the amount of iron in northeastern local vegetables.
2. To determine the amount of vitamin C which was expected to enhance iron absorption in northeastern local vegetables.
3. To determine the amount of phytate and crude fiber which were expected to inhibit iron absorption in northeastern local vegetables.
4. To study the relationship between the amount of iron, vitamin C, phytate and crude fiber in northeastern local vegetables.
5. To estimate the bioavailability of iron from iron, vitamin C and phytate contents in northeastern local vegetables.

CHAPTER II

LITERATURE REVIEW

1. Iron

Iron deficiency anemia is a wide spread nutrition problem in many parts of the world. It is widely recognized that anemia and iron deficiency are highly prevalent in developing countries, especially in young children, pregnant and lactating women. In these risk groups the iron requirement are higher compared to their energy requirements than in other population groups, so they are especially vulnerable to iron deficiency anemia. According to the Interdepartmental Committee on Nutrition for National Defense (ICNND) nutritional survey in 1962, 25.4 percent and 52.6 percent of 252 Thai members of the military and 209 civilians were found to be anemic (ICNND, 1962). The report showed that 12 percent of the members of the military and 22 percent of the civilians (both sexes, school children and adults) had hemoglobin concentrations below 10 g/dl.

Migasena *et al.* (1972) reported that 37 percent of village preschool children in Northeast Thailand and 32 percent orphan preschool children in Bangkok were anemic. Valyasevi *et al.* (1974) found that 33 percent infants up to 2 years and 10 percent children of 2 to 6 years had anemia in Central and Northeast Thailand.

In rural area, the size of anemic problem was quite magnitude particularly in preschool children as show in a wide range of 10-40 percent and the highest prevalence was found among the infants under 2 years of age. From this study, 2 groups of children from Bangkok and Khonkaen were revealed that 30.2 percent and 61.1 percent being anemic respectively (Areekul *et al.*, 1972).

Areekul *et al.* (1976) studied the prevalence of anemia among pregnant women attending Siriraj Hospital and found that 16-22 percent of them being anemic by hemoglobin concentration below 11 grams percent and about 25.5 percent of anemic women revealing low serum iron levels and percent of transferrin saturation.

Wasi *et al.* (1973) studied the anemia in 13 provinces of 4 parts of Thailand in 1973 and found that there were no difference in the prevalence of anemia between men and women, 35 percent of male and female population. However, the highest incidence was observed in the northeast region. They classified hematocrit values of below 40 percent in males and below 35 percent in females as anemic status. By this criteria 11 to 94 percent of the population in different areas of Thailand were anemic, which suggested that iron deficiency anemia was one of the most important nutrition problems in Thailand.

1.1 Causation of Iron Deficiency Anemia

This form of anemia is characterized by a reduced concentration of hemoglobin in the blood and a depletion of total body iron content. The three causes of iron deficiency anemia are (1) chronic blood loss, such as from a chronically bleeding peptic ulcer, bleeding hemorrhoids or from parasites (hookworm disease) or malignancy, (2) inadequate iron intake or absorption and (3) increased iron requirement for growth of blood volume, which occurs in infancy, puberty, pregnancy and lactation. Another cause of iron deficiency anemia is defective release of iron into the plasma from the iron stores as occurs in chronic inflammation and other chronic disorders (Shils and Young, 1988).

Increased Losses

The major factor affecting iron requirement is the amount of iron loss. It has been well established that hookworm produces blood loss which leading to iron loss. The study on 2 groups of children with ages ranging from 7 to 10 years in Bangkok and Khonkaen revealed the prevalence of iron deficiency anemia being significantly higher in children in Khonkaen than that of children in Bangkok (Areekul *et al.*, 1972).

Two studies were performed in 162 school children and 590 hookworm patients whom were admitted to the hospital. The results showed that the hemoglobin in the non-infested group was significantly higher than that of the group with worm load representing by 2,000 eggs per gram faeces

in adults and 4,000 eggs per gram faeces in children (Areekul *et al.*, 1972; Areekul, 1979).

Triteeraprapab and Nuchprayoon (1998) studied the prevalence and relationship between eosinophilia, anemia and parasitism in 169 Thai-Karens from Mae Lamung and Mae Chan subdistricts, Umphang district, Tak Province. They found that Mae Chan residents had high prevalence of anemia (50 percent) as well as high prevalence of parasitic infections, particularly hookworm infection (59 percent), while Mae Lamung subjects had anemia and 25 percent had hookworm infection.

Decreased Absorption and Iron Intake

It is evident that iron deficiency is still prevalent in Thailand even though the intake of iron is 10-23 mg/day. This possible be due to the fact that the iron in some diets is not readily available for absorption either the chemical form or the presence of substances that inhibiting absorption such as phytate and crude fiber. A study on intake of rice, vegetables and spices which containing almost 10 mg iron showed absorption approximately 0.4 percent in normal subjects (Hallberg *et al.*, 1974).

The intake of iron in Thai diet has been studied by many authors. Chandrapanond, Ratchatasilpin and Tansuphasiri (1972) reported a mean intake of 11.9 mg iron/ person/day by the chemical analysis method. The food iron intake per day by farmers in Bangkok, Ang Thong and Si Sa Ket were found to be 24.20 mg/day, 13.17 mg/day and 7.37 mg/day, respectively (Pattanachak *et al.*, 1981). It can be seen that the amount of iron intake of the

extremely poor rural population in northeastern Thailand is virtually much lower than those of people in Bangkok and Ang Thong farmers in central Thailand. It is therefore understandable that such limited iron intake would be the prime factor, and despite the body's efforts to gain a higher percentage of iron absorption, may give rise to less or inadequate iron for erythropoiesis. The supply of iron is indeed very marginal. There is a high likelihood that many people especially pregnant women will fall victim to iron deficiency.

Increased Requirements

To maintain nutritional balance, the daily intake of iron must replenish the amount lost from the body plus supplying any additional amount needed for growth and development. The increased requirements may be physiological or pathological. Normally, there is a certain percentage of women losing large qualities of blood during menstruation. During infancy, a close relationship exists between diet and iron deficiency. At puberty, as well as in infancy, there is an acceleration of growth and an increased requirement for iron (Shils and Young, 1988).

1.2 Human Requirement and Basis for RDI

The total amount of iron in the body is about 3 to 5 g in adult (Czajka-Narins, 1996). Since iron is continuously recycled in body, the daily iron requirement is relatively small. The amounts of iron would be replaced for the lost from the body and necessary for growth and development.

Requirements for adults ages 20-50 year are about 14 μg iron/kg body weight (0.25 μmol/kg) for males and about 22 μg (0.39 μmol/kg) for premenopausal females (Herbert, 1987). If the average absorption of food iron is 10 percent, an allowance of 10 mg/d is recommended to replace the average loss of 1 mg/d in adult males (Green *et al.*, 1968) and postmenopausal females (Table 1). The allowance for women of childbearing age is set at 15 mg/d to meet the additional needs imposed by menstruation.

Pregnant women need iron to replace basal losses, to allow an average of 450 mg for expansion of the red cell mass, and to provide about 290 mg iron to the term fetus and about 25 mg to placenta (American Medical Association Committee on Iron Deficiency, 1968). Iron needs of lactating women are not substantially different from those of nonpregnant women (see Table 1).

The normal term infant can maintain satisfactory hemoglobin levels from human milk without other iron sources for the first 3 month of life. Starting about the 3rd month of life and continuing to age 3, infant require a daily iron intake of 1 mg/kg/d to a maximum of 15 mg; 10 mg/d is usually adequate (American Academy of Pediatrics Committee on Nutrition, 1976; Craig, Balbach and Vyhmeister, 1984).

Children and adolescents need iron, not only to maintain hemoglobin concentrations, but also to increase their total iron mass during the period of growth. The increases in iron mass related to growth in body size, children and adolescents require as much iron as adult men (Dallman, Siimes

and Stekel, 1980). To attain a target iron storage level of 500 mg for both sexes by age 20-25, an allowance of 10 mg/d is recommended for children (Herbert, 1987).

Table 1. Recommended Daily Intakes for Iron (RDI) (from American Medical Association Committee on Iron Deficiency, 1968)

Category	Age	RDI (mg/d)	Category	Age	RDI (mg/d)
Infants	0-2.9 mo	*	Females	10-14.9 yr	15
	3-5.9 mo	6.6		15-17.9 yr	15
	6-11.9 mo	8.8		18-24.9 yr	15
Children	1-1.9 yr	10		25-49.9 yr	15
	2-5.9 yr	10		50-69.9 yr	10
	6-9.9 yr	10		70+ yr	10
Male	10-11.9 yr	12	Pregnancy	0-2.9 mo	15+30
	12-17.9 yr	12		3-5.9 mo	15+30
	18-24.9 yr	10	Lactation	6-9 mo	15+30
	25-49.9 yr	10		0-5.9 mo	15
	50-69.9 yr	10		6+ mo	15
	70+ yr	10			

* Storage iron present at birth, normal term infant does not require exogenous iron beyond that provided by breast milk or formulas containing iron of bioavailability equivalent to that of breast milk for the first 3 month of life.

The elderly are at risk of developing poor body stores of iron. Survey data suggested that inflammatory disease, rather than iron deficiency, was the main cause of anemia in the elderly (Roebothan and Chandra, 1996; Yip, Johnson and Dallman, 1984). Additionally, after the menstrual years, the daily iron requirement of women approximates that of men (Table 1), and the iron RDI recommended for the elderly is the same as that for adult males.

1.3 Recommended Dietary Allowance of Iron

The Food and Nutrition Board of United Stated has recommended a daily intake of 10 mg of iron for men and postmenopausal women. An intake of 15 mg/d is recommended for women during child-bearing years to replace the losses of menstruation and to provide for iron stores sufficient to support a pregnancy. Female adolescent requirements are also set at 15 mg to provide for the needs of rapid growth. RDA for teenage male is 12 mg/d (Table 2) (Food and Nutrition Board, 1989).

Table 2. Recommended Dietary Allowances for Iron (from National Academy of Science, 1989)

Category	Age (years)	RDA (mg)
Infants	0.0-0.5	6
	0.5-1.0	10
Children	1-3	10
	4-10	10
Males	11-18	12
	19-50	10
	51+	10
Females	11-18	15
	19-50	15
	51+	10
Pregnant		30
Lactating	1^{st} 6 months	15
	2^{nd} 6 months	15

1.4 Assessment of Iron Status

Anemia is the end result of severe iron deficiency. The homeostatic mechanisms of the body maintain hemoglobin levels at the expense of body stores. The overall iron status of an individual is related to the dietary intake, the proportion absorbed, the tissue stores, the body's requirements and body losses. Cook and Finch (1979) concluded that the cause of anemia could reasonably be attributed to iron deficiency only when at least

two of these iron parameters fall within the deficient range. It may be best to perform all four tests-serum ferritin (SF), transferrin saturation (TS), free erythrocyte protoporphyrin (FEP), and hemoglobin (HGB)-when assessing the iron status of population. Serum or plasma ferritin is the most sensitive parameter of iron status. It falls only with true iron deficiency, whereas transferrin saturation, free erythrocyte protoporphyrin and hemoglobin levels are affected by chronic infection and other factors that may cause an anemia that looks like iron deficiency anemia when in fact iron is adequate.

Each of these four parameters of iron status reflects change in different body iron compartments and is affected at different levels of iron depletion. It is convenient to define three stages of iron deficiency (Figure 1). The least severe stage of iron depletion, defined as iron stores less than 100 mg, is identified by a SF level less than 12 μg/liter only the SF is affected at this stage of iron lack. With continued iron loss, iron stores become exhausted and the second stage of iron deficient erythropoiesis ensues which is recognized by a fall in the TS and/or a rise in FEB. In the final stage red cell production becomes further impaired and iron deficiency anemia can be identified by a significant fall in the circulating hemoglobin (Cook and Finch, 1979)

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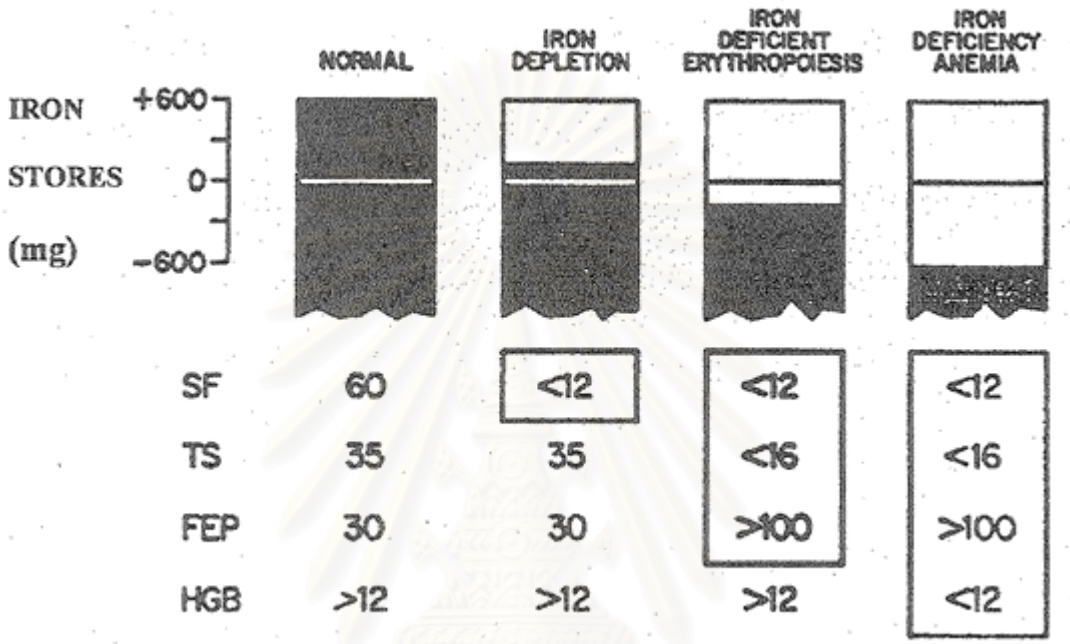


Figure 1. Parameters of iron status in relationship to body iron stores (Cook and Finch, 1979).

1.5 Forms of Iron in Diet

There are two types of dietary iron based on different mechanisms of absorption: heme iron and nonheme iron.

Heme iron

Heme iron is present in hemoglobin and myoglobin and is absorbed into the mucosal cells as the intact iron porphyrin complex from which the iron is released intracellularly by a specific enzyme. Heme iron forms only 10 to 15 percent of the total iron intake in most Western diets but is still important as it usually has a higher bioavailability than nonheme iron (Hallberg, 1981a).

Heme iron, found only in meat, fish, and poultry (MFP), has a much higher bioavailability ranging from 15 to 35 percent (Monsen and Balintfy, 1982), and is not affected by other dietary constituents. However, heme iron in raw MFP vary from 42 percent in chicken breast meat, to greater than 90 percent in red beef (Carpenter and Clark, 1995; Chen *et al.*, 1984).

Nonheme iron

Nonheme iron is the main part of dietary iron and most of it comes from cereals, vegetables, fruits, eggs and iron used for fortification of foods. The nonheme iron forms the main part of iron, about 85 to 90 percent, in the Western type diets. About half of the iron content of meat products is nonheme iron (Hallberg, 1981a). Nonheme iron has a low bioavailability ranging from 2 to 20 percent (Monsen and Balintfy, 1982), and is influenced

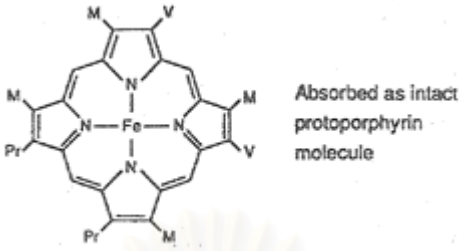
by some food components both decreasing effect, e.g. phytate and crude fiber and enhancing effect, e.g. vitamin C (Monsen, 1988).

1.6 Mechanisms for Absorption of Iron

The two forms of food iron have startlingly different proposed mechanisms for absorption (Figure 2). In the gastrointestinal tract, heme appears to be freed from associated compounds, such as globin. Then, as an intact protoporphyrin molecule, it is absorbed into the mucosal cell, where the iron is released and enters the body iron pool. In human being, the absorption of heme is an efficient process (Monsen, 1988).

Nonheme iron is associated with a variety of dietary compounds. During digestion, nonheme iron is freed and rapidly forms chelates. Depending upon the nature of the iron:chelate complex, one of two paths is predicted. If the iron:chelate maintains solubility and the chelate can release iron, the iron may be absorbed as the iron:chelate approaches the gut mucosa and unchelates. On the other hand, if the iron:chelate is strongly bonded, insoluble, and remains chelated, the iron:chelate will be excreted. Ascorbic acid, other organic acids, amino acids, and certain intermediary products of digestion, such as are formed in meat/fish/poultry (MFP) digestion, are examples of chelates that enhance nonheme iron absorption. Certain other products of digestion (e.g., calcium phosphate aggregates, tannates, and EDTA) are among chelates that encourage the excretion of nonheme iron (Monsen, 1988).

I. HEME IRON



II. NONHEME IRON

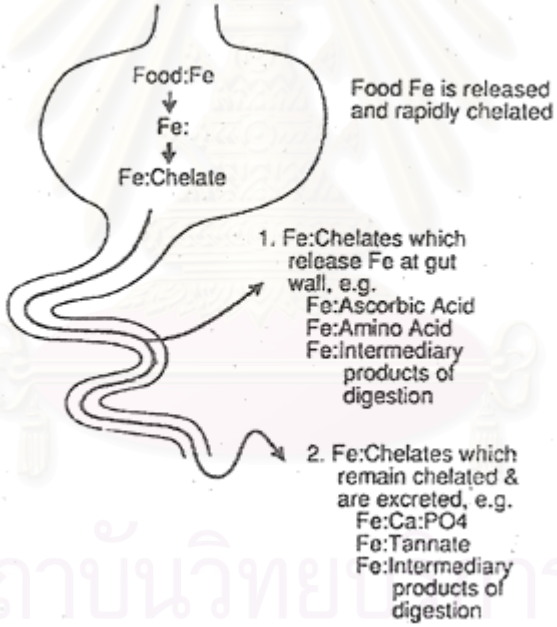


Figure 2. Schematic diagram of the absorption of two forms of food iron: heme and nonheme iron (Monsen, 1988)

1.7 Dietary Factors Affecting Iron Absorption

Nonheme iron absorption has been shown to be affected by several factors in the diet. Vitamin C increases the absorption markedly. This increase is obtained both by adding vitamin C as such or by adding foods with a high content of vitamin C. The increasing is over a wide range related to the amount of vitamin C added. Certain foods have been found to decrease the absorption especially phytates and crude fiber (Rossander *et al.*, 1979).

The amount of potentially available iron from food depends not only upon the amount of iron supplied but also the nature form of that iron and the composition of the meal which is consumed (Narasinga and Prabhavathi, 1978). The total iron in meal is thus the relatively poor indicator of adequacy of iron requirement. The proportion of heme to nonheme iron is concerned to the amount of absorbable iron. The complex meals are composed of a variety of foods which their components are enhancing and inhibiting iron absorption.

2. Vitamin C

Ascorbic acid is a strong promoter of nonheme iron absorption, as shown in several studies. The effect of ascorbic acid on iron absorption may be due to its ability to form soluble complexes with ferric ions and to its reducing action on iron, which produces the more soluble ferrous form. Ferrous ion is better absorbed than is ferric ion (Hallberg, 1981b).

In an extensive study by Cook and Monsen (1977) in which 6 different amounts of ascorbic acid (25-1000 mg) were added to semisynthetic meal, a strong relation was seen between log amounts of ascorbic acid and the log absorption ratio ($r^2 = 0.958$; $n = 25$).

Layrisse *et al.* (1974) demonstrated a 5-fold increase in iron absorption from maize when taken with 70 mg of ascorbic acid. Addition of 100 mg ascorbic acid to the semisynthetic liquid formula increased iron absorption 4.14 times, whereas addition of the same amount of ascorbic acid to a so-called standard meal containing meat, potatoes, and milk increased iron absorption 2.77 times. In another study from the same group, addition of 100 mg ascorbic acid to another but similar liquid formula containing 85 mg phytate-P increased iron absorption 3.14 times (Reddy *et al.*, 1996).

Cook *et al.* (1997) studied the enhancing effect on iron absorption of adding 50 mg ascorbic acid to four unfortified cereal foods (maize, wheat, quinoa and rice). They found that the mean absorption of iron was increased to 270 percent for all cereals.

These findings suggested that the ability of ascorbic acid to reduce iron and thus to prevent the formation of less-soluble ferric compounds was probably an important mechanism of action for the absorption-promoting effect of ascorbic acid. An enhancing effects of ascorbic acid on iron absorption, however, was also seen in the absence of phytate and polyphenols. Addition of 50 mg ascorbic acid, for example, to wheat rolls with no detectable phytate increased mean iron absorption from 22.4 to 37.6 percent (Hallberg *et al.,* 1989).

Tuntawiroon *et al.* (1991) showed that the addition of 100 mg ascorbic acid reduced inhibition of iron absorption from 5 g Yod Kratin (*Leucaena glauca*) by half and the inhibition from 10 g Yod Kratin (*Leucaena glauca*) by a quarter.

Studies suggested that equal to or greater than 50 mg ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing greater than 100 mg tannic acid (Siegenberg *et al.*, 1991).

Rossander *et al.* (1979) tested the impact of adding 150 ml of orange juice to a breakfast consisting of bread, butter, marmalade, cheese and coffee. Orange juice increased iron absorption from 3.7 to 8 percent. When tea replaced coffee in breakfast meal, the enhancing effect of the orange juice was less pronounced.

Gillooly *et al.* (1983) studied the effects of organic acids on the absorption of iron from vegetables. They found that 15 mg ascorbic acid improved the geometric mean iron absorption from the basic meal from 0.031 to 0.081. The later study showed that when only an equimolar amount with the iron (9.5 mg) was added, geometric mean iron absorption increased from 0.024 to 0.063 (Gillooly *et al.*, 1984).

Davidsson *et al.* (2001) showed the geometric mean iron absorption was about 5 percent from the test meals that were fortified with 14 mg iron and contained ascorbic acid at a relatively low molar ratio relative to fortification iron (0.6:1). When they increased the ascorbic acid content to 70 mg (resulting in a molar ratio of 1.6:1), mean iron absorption increased significantly to 8.2 percent.

Cook *et al.* (1972) provided a meal of maize with 500 mg. ascorbic acid and found that absorption of iron was 6 times higher than basal diet. The addition of 50 or 100 mg ascorbic acid to maize-meal porridge caused approximately a 10-fold increase in iron absorption. The increase was much less when tea was present, being 2-fold and 5-fold with 50 and 100 mg ascorbic acid, respectively. The inhibitory effect of tea on iron absorption could, however, be overcome by giving larger doses of ascorbic acid (250 and 500 mg) (Derman *et al.*, 1977).

Ballot *et al.* (1987) studied the effects of fruit juices and fruits on the absorption of iron from a rice meal. They found a close correlation between iron absorption and ascorbic acid content of the fruits. The corrected geometric mean iron absorption from 100 ml orange juice (contained 28 mg ascorbic acid) was 0.139, while geometric mean iron absorption from unsupplemented basal rice meal was only 0.025. Absorption of 3 to 4 mg vegetal iron was increased about twice by 50 mg of meat, about thrice by 100 g of fish and about five times by 150 g of papaya containing 66 mg of ascorbic acid (Layrisse *et al.*, 1974).

MacFarlane *et al.* (1988) studied in nuts and found that addition of 25 and 50 mg ascorbic acid to bread and peanut meal would improve iron absorption from 3.1 to 5.2 and 10.7 percent, respectively.

3. Phytate

Phytic acid, commonly called myo-inositol hexaphosphoric acid or, scientifically, 1,2,3,4,5,6-hexakis (dihydrogen phosphate) myo-inositol (IUPAC-IUB, 1968). Phytic acid exists wholly as a soluble salt. This organic salt reduces the absorption of divalent and trivalent cation (Zn^{++}, Ca^{++}, Mg^{++}, Fe^{++}, and Fe^{+++}) by combining with them to form insoluble salt. This make reduction of their availability for absorption (Maga, 1982). Hence, phytic acid has been recognized as undesirable compound in spite of its high phosphorus content. The structure formula of phytic acid is shown in Figure 3 (Wheeler and Ferrel, 1971).

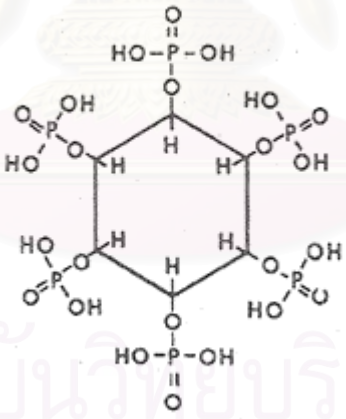


Figure 3. Structure of phytic acid (Wheeler and Ferrel, 1971)

3.1 Phytic acid in foods

Phytic acid is found in plant based foods such as unrefined cereals, seeds, nuts, legumes, tubers, some fruits and vegetables (Oberleas and Harland, 1981). Phytates have been found in cereal grains and legumes up to a level of approximately 5 percent by weight. Sesame seeds have been reported to contain the highest level of phytate (5.18 percent) (De Boland, Garner and O'Dell, 1975).

Oberleas (1973) investigated the phytate content in some popular plants. He found that green beans, carrots and broccoli contained trace amount of phytate while lettuces, onions, mushroom and spinach contained too small amount to be detected. In contrast, potatoes, sweet potatoes and artichokes had moderate amounts, while cereals, nuts and legumes contained even higher level.

The phytate content was determined in various Thai plants which classified by their edible part into 5 groups : 1) cereal grains 2) pulses, nuts and seeds 3) leafy vegetables 4) flower or fruit-consumed vegetables 5) starchy roots, tubers and young shoots. The result showed that the mean of phytate content in the group of pulse, nut and seed was the highest. The lower phytate content was found in cereal grain, leafy vegetable, flower or fruit-consumed vegetable as well as group of starchy root, tuber and young shoot (Boontaveeyuwat, Boonmongkol and Boonpikum, 1990).

The staple foods in Thailand are cereal based, including most notably rice. In addition, certain high protein legumes and oily seeds are among the food items being promoted as supplementary foods for population groups experiencing or at high risk of developing protein energy malnutrition. Common ingredients used to prepare supplementary food include soybean chips, mixed supplementary food containing rice, bean and sesame seeds, and other fermented foods. Of these ingredients, white and black sesame seeds contain the highest phytate levels (2.4 and 2.0 percent respectively). Other items containing considerably high levels of phytate are soybean, peanut, cowpea, mungbean, and rice bean (1.4, 1.1, 0.8, 0.8 and 0.7 percent, respectively) (Nititham and Srianujata, 1991).

Srianujata and Nititham (1992) studied the effects of cooking processes on phytate levels in selected Thai foods. This study demonstrated cooking processes (i.e., boiling, steaming, frying, roasting), effected phytate levels in certain legumes as well as sesame seed. Specifically, frying and roasting reduced phytate levels to a greater extent than boiling and steaming, which have little effect. Prepared foods were found to contain low to moderate phytate levels, except for fried soybean curd which contained a considerably high phytate level.

3.2 Effect of phytate on iron absorption

Numerous studies have led to the conclusion that phytic acid and its derivatives can bind essential dietary minerals, thus making them unavailable or only partially available for absorption. This problem becomes especially important when iron is one of the cation that binding effect can occur.

McCance and Widdowson (1943) showed that iron absorption from white bread was inhibited by incorporation with sodium phytate which interfered with absorption of ferrous and ferric iron. The study of Gillooly *et al.* (1984) found that 2 g of sodium phytate added to a broccoli meal resulted in decreasing of the geometric mean iron absorption from 0.185 to 0.037.

Hallberg (1987) who studied the effects of phytate on the absorption of iron, found a strong semilogarithmic relationship (r = 0.99) between the inhibition of iron absorption and the amount of phytates. As little as 5-10 mg phytate phosphorus, added to a wheat roll containing 3 mg iron, inhibited iron absorption by 50 percent.

The inhibition of iron absorption was strongly related to the amount of phytate added; 2 mg inhibited absorption by 18 percent, 25 mg by 64 percent, and 250 mg by 82 percent (Hallberg *et al.*, 1989).

Rossander-Hulthen, Gleerup and Hallberg (1990) showed that oat products markedly inhibited the absorption of nonheme iron. The serving of oat porridge and oat bran to a continental type of breakfast reduced the fractional iron absorption by about 60 percent and 45 percent respectively. The inhibition could be explained by the high phytate content of oat products.

Tuntawiroon *et al.* (1990) studied the effect of the phytate content in rice on iron absorption. They found that the bioavailability of iron in a meal composed of meat, a vegetable and rice varied about three fold (22.1-7.5 percent) depending on the different phytate content of the rice (4 levels between 35 and 175 mg phytate were studied). From this study, iron absorption decreased successively when the rice had a higher phytate content.

Hurrell *et al.* (1992) investigated the effect of reducing the phytate in soy-protein isolates on nonheme iron absorption in human subjects. Iron absorption increased fourfold to fivefold when phytic acid was reduced from its native amount of 4.9-8.4 mg/g to less than 0.01 mg/g of isolate.

4. Fiber

Fiber content of foods has been described in terms of crude fiber, determined by subjecting materials to digestion by acid and alkali. Crude fiber content is an index of the amount of indigestible matter, or roughage, in a foodstuff. It is made up of cellulose, lignin, and hemicelluloses. Although fiber has no appreciable food value, it functions in the intestinal tract to give bulk to the contents and so stimulates intestinal peristalsis. Whereas, indigestible dietary fiber or ‘roughage’ is made up of hemicellulose, pectic substances, gums, mucilages, cellulose and lignin as well as undigested protein and lipid and ingested filth (Kirk and Sawyer, 1991).

The actual action of digestive enzymes is less rigorous. The amount of fiber remaining after digestion in the human alimentary tract is considerably greater than that estimated by the crude fiber determination process. The dietary fiber contents as presently measured are usually two to five times higher than those for crude fiber. However, no correction factors can be applied because the relationship between the two kinds of fiber varies depending on the composition of particular foods. Bran flakes, for example, contain six times as much dietary fiber as crude fiber, but in strawberries the amount of dietary fiber is only 1.6 times greater than crude fiber (Marlett, 1992).

4.1 Fiber in foods

Puwastien, Valaipatchara and Kongkachuichai (1990) investigated dietary fiber content in common Thai food. They found that plant with high protein content such as different varieties of pulses and sesame seeds provided a high content of dietary fiber, 19-28 g per 100 g sample, whereas that of sunflower seed contained 12 percent dietary fiber. Low concentration of dietary fiber (0.7-2 percent) was found in different varieties of rice, the staple food of Thai people. Moreover, a wide variation of dietary fiber content was found in different kinds of vegetables, ranging from 1.0 g in 100 g angled-type gourd (‘Buablium’) to 13.6 g in 100 g eggplant (‘Makuapuang’). Likewise wide variation of the amounts of total dietary fiber were found in fruits. According to the high water content, fruits contained lower dietary fiber than vegetables, ranging from 0.3 g in 100 g watermelon to 8 g in 100 g sapodilla (‘Lamud’).

4.2 Effect of fiber on iron absorption

The decrease in iron absorption affected by fiber was explained by several studies. Fernandez and Phillips (1982) described physicochemical interactions among ferrous iron and the components of fiber. They concluded that fiber was able to bind iron undercertain circumstances and to a degree whereby intestinal absorption of dietary iron might be impaired.

Olszen *et al.* (1978) showed that fiber might interfere with iron absorption by several actions. The increase in weight and bulk of undigested residues in the gut due to the higher intake of fibers lead to decrease transit times and increase frequency of defecation. Fecal weights was increased. The time of contact between villi and iron decreases with lessening opportunity for absorption.

Effect of pH upon the binding of iron by fiber was demonstrated by several investigators. Thompson and Weber (1979) studied the binding of iron to various fiber sources including wheat bran, corn bran, soy bran, oat hulls, rice bran and cellulose under conditions which parallel the physiological pH of the stomach and duodenum. These workers found that while the endogenous minerals were not bound to the fibers at the acidic pH, they were rebound when the pH was raised.

Reinhold, Ismail-Beigi and Faraji (1975) showed that fiber of wheat formed stable complexes with iron and also zinc and calcium. The metals combining with protein or wheat starch were released during digestion with peptidases and amylases. In contrast fiber, being resistant to digestive secretions, retained bound metal intact. Ismail-Beigi, Faraji and Reinhold (1977) showed that cellulose formed some sources, certain cellulose derivatives and hemicelluloses prepared from wheat bran shared the ability to bind iron. Fiber might promote the conversion of ferrous iron to ferric iron in some circumstances. Such a conversion would tend to decreased iron availability.

Simpson, Morris and Cook (1981) showed that muffins containing 12 g of bran reduced iron absorption by 74 percent when added to a meal of low iron availability and by 51 to 58 percent when added to meal containing either meat or ascorbic acid.

Dobbs and Baird (1977) showed that the percentage of iron absorbed from white bread was considerably higher than that from wholemeal bread. Serum iron concentrations was lower following ingestion of wheat bran or wholemeal bread as compared with white bread (Jenkins, Hill and Cummings, 1975; Persson, Raby and Fonns-Bech, 1975).

Cook *et al.* (1983) studied the effect of fiber on the absorption of food iron by multiple radioiron absorption tested in normal subjects. In an initial study, the effects of purified fiber sources pectin and cellulose were compared with that of bran and that of wheat flour in a muffin meal. The mean absorption averaged 2.26 percent for plain muffins, 1.07 percent for bran muffins, 1.89 percent for pectin muffins and 2.26 percent for cellulose muffins. Only the effect of bran was statistically significant. Other study was performed to determine the effect of naturally occurring fiber on the absorption of nonheme iron from two complete meals. Mean absorption from the low-fiber and high-fiber meals were significantly different, averaging 6.07 percent and 2.96 percent, respectively.

5. Techniques for measuring the bioavailability of dietary iron

Low iron availability is considered to be one of the most significant factors in the etiology of iron deficiency. The absorption of nonheme iron from foods is influenced by a variety of inhibitors and promotors of iron absorption (Rossander *et al.*, 1979). Therefore, in evaluating the causes of iron deficiency, it is important to consider both the iron content and the availability of dietary iron.

5.1 *In vivo* methods

The most reliable method for determining bioavailability of iron from diets is to measure iron absorption in humans using the extrinsic tag method. In the extrinsic tag method a trace amount of radiolabeled iron, usually $^{59}FeCl_3$ or $^{59}FeSO_4$, is added to a test diet. Bioavailability is measured by extrapolating the amount of radioiron absorbed to the quantity of iron dosed (Cook *et al.*, 1972). An alternate animal model to determine iron availability from human diets has been recently proposed (Narasinga, Siva Prasad and Vijayasarathy, 1977). These *in vivo* methods are accurate, however, they are time consuming and expensive for screening large numbers of food material.

5.2 *In vitro* methods

An *in vitro* method, on the other hand, have several advantages for rapid screening and testing of iron availability from diets consumed and suggest improvements in the diets so as to increase availability of iron from them. Ionizable iron determined by ∝, ∝′-dipyridyl had been suggested earlier to represent available or ionizable iron (Shackleton and McCance, 1936). This method was proved to be of little value, as it did not take into account the conditions in the stomach and small intestine which influence iron availability. Ranhotra, Hepburn and Bradley (1971) have proposed a method for determine available iron based on the estimation of iron released from foods treated with pepsin-HCl solution simulating gastric juice. However, most of the dietary iron is absorbed from the small intestine and not from the stomach, this method does not determine the true availability of iron.

5.3 Calculation of iron bioavailability

Several attempts have been made to devise algorithms to estimate the bioavailability of dietary iron content of meals. Monsen *et al.* (1978) have proposed a method for calculating absorbable iron. The method is based on the assumptions that dietary iron is mostly found as either heme or nonheme iron and that nonheme iron is absorbed at a very low level unless enhancing factors are present in the meal. For each snack or meal, the amounts of total iron, heme iron, nonheme iron, and enhancing factors are included in the calculation.

Percentage absorption of heme iron is influenced by iron status in an inverse logarithmic function. In subjects with 0, 250, 500, and 1000 mg of iron stores, absorption from heme iron is estimated to be 35, 28, 23, and 15 percent, respectively. An individual with no iron stores may be expected to absorb about 35 percent of the heme iron and 8 percent of nonheme iron (if enhancers are present) in the diet, while an individual with replete iron stores (1000 mg) would probably absorb only 15 percent of heme iron and 3 percent of nonheme iron (with no enhancers present). The rate of absorption of nonheme iron is between 3 and 8 percent, depending upon the number of enhancing factors (Monsen *et al.*, 1978; Shils and Young, 1988).

The method proposed to estimate the quantity of absorbable iron in a given meal requires computation of 5 sums: 1) total iron, 2) heme iron, 3) nonheme iron, 4) ascorbic acid, and 5) meat, poultry, and fish. Iron in a meal can then be classified as having high, medium, or low availability. It is suggested that the reference level for body iron stores of 500 mg be used for calculations (Monsen *et al.*, 1978; Monsen and Balintfy, 1982).

Monsen *et al.* (1978) prepared for 4 meals to illustrate the effects of ascorbic acid and meat tissues on the estimated amount of iron absorbed. Iron absorption from the low availability nonmeat tissue containing meal was estimated to be 0.15 mg and from the medium availability meal containing meat, poultry, or fish to be 0.29 mg of iron. In contrast, the estimated iron absorption was two to four times higher from the high availability meals, one containing meat, poultry, or fish and the other without meal tissue. The basic assumptions in this study are: 1) an average of 1.4 mg iron/day will be

required to be absorbed by the menstruating woman to replace physiologic losses and that 2.8 mg/day will be required to replace the total body iron losses of up to 95 percent of menstruating women; 2) an inverse relationship exists between iron stores and absorption so that the higher iron stores, the lower will be the percent of available iron absorbed; 3) iron stores of about 500 mg are desirable for women to meet the requirements of pregnancy without supplement iron.

Recently, Tseng *et al.* (1997) refined the model by including inhibitors found in Russian diets. The contents of animal tissue, nonheme iron, vitamin C, phytate and tea were included for estimating nonheme iron absorption. The interactions occur in gut at the time of food ingestion, the analysis of iron bioavailability requires information on total dietary intakes for each individual in the sample for each eating occasion. The steps taken in adjusting for iron bioavailability are shown schematically in Figure 4 (Tseng *et al.*, 1997).

In Figure 4, adjustment for iron availability, the iron content of each food was first separated into its heme and nonheme components. The assumed proportion of total iron in each food that was nonheme was 60 percent for meats and 100 percent for nonmeats; any remaining iron was assumed to be heme. Next, heme iron availability was assumed to be 23 percent. Nonheme iron availability was adjusted for meat, fish and poultry (MFP) and vitamin C based on algorithms taken from Monsen *et al.* (1978) and Monsen and Balintfy (1982) and ranged from 3 to 8 percent. Further adjustments to available nonheme iron were made based on the amounts of

tea and phytates consumed in the meal. Nonheme iron availability was reduced by 40 percent when the amount of tea consumed in the meal exceeded 225 g. For additional adjustment of nonheme iron availability for the effect of phytate consumed in the same meal was performed using method developed by Hallberg *et al.* (1989). Thus, the known amount of food iron bioavailability would be useful to more precisely plan the adequate dietary iron intake.

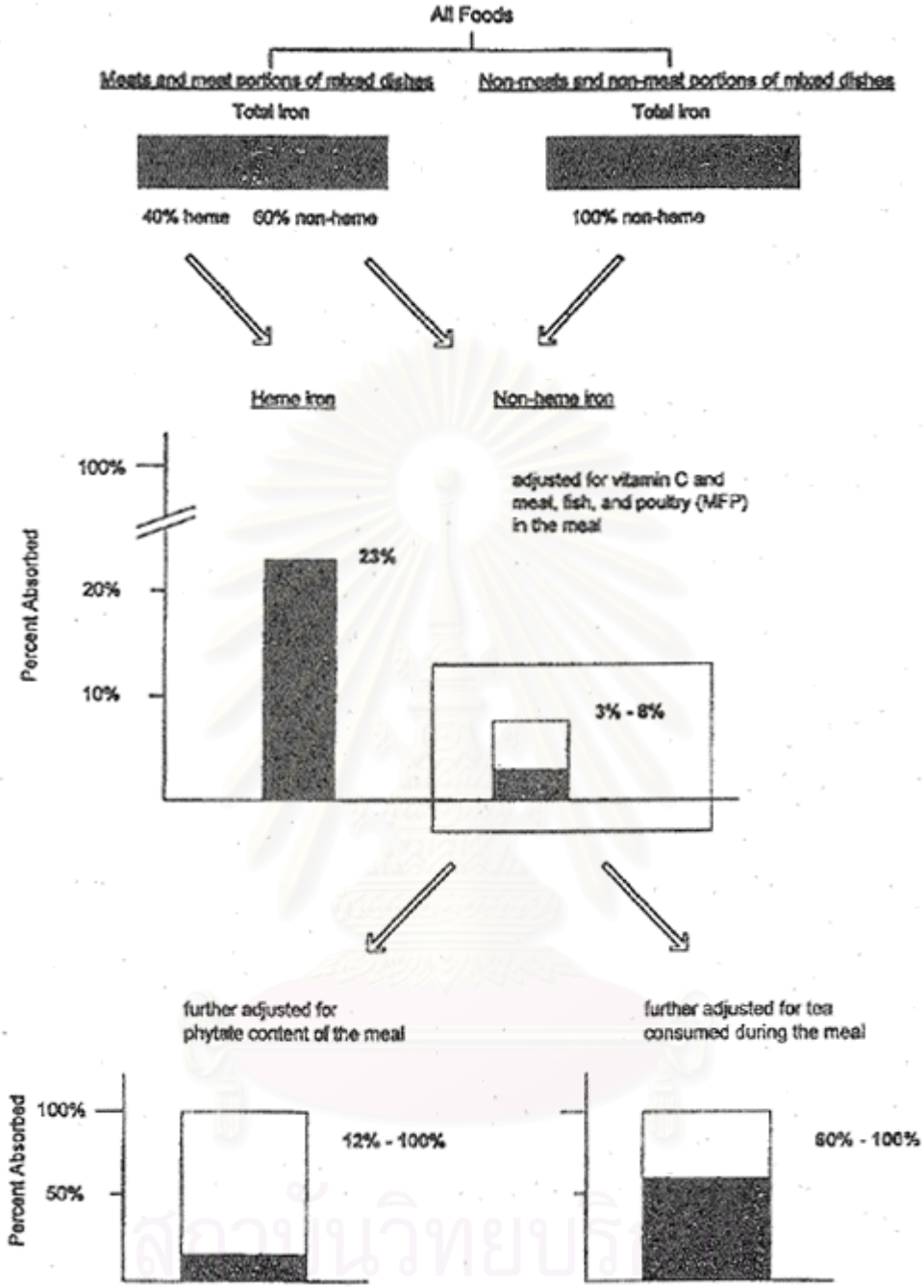


Figure 4. Steps taken to adjust for iron bioavailability (Tseng *et al.*, 1997)

CHAPTER III

MATERIAL AND METHOD

Material

1. Samples preparation

The samples which were picked up during rainy and hot seasons were purchased from various provinces in northeastern Thailand (Khonkaen, Loei, Kalasin, Udonthani and Mahasarakam). Thirty-two kinds, commonly used and could be purchased from the markets, were selected as samples for this study (see appendix A).

They were washed with tap water for a short time in order to remove soil and remove most of water with soft paper. Each sample was divided into 2 portions. The first portion was cut into small pieces and finely ground. Moisture and vitamin C contents were determined on the fresh samples. The second portion was dried at 50 – 60°C for 1 to 2 days, ground and stored in a desiccator for determination on content of iron, phytate and crude fiber (Figure 5).

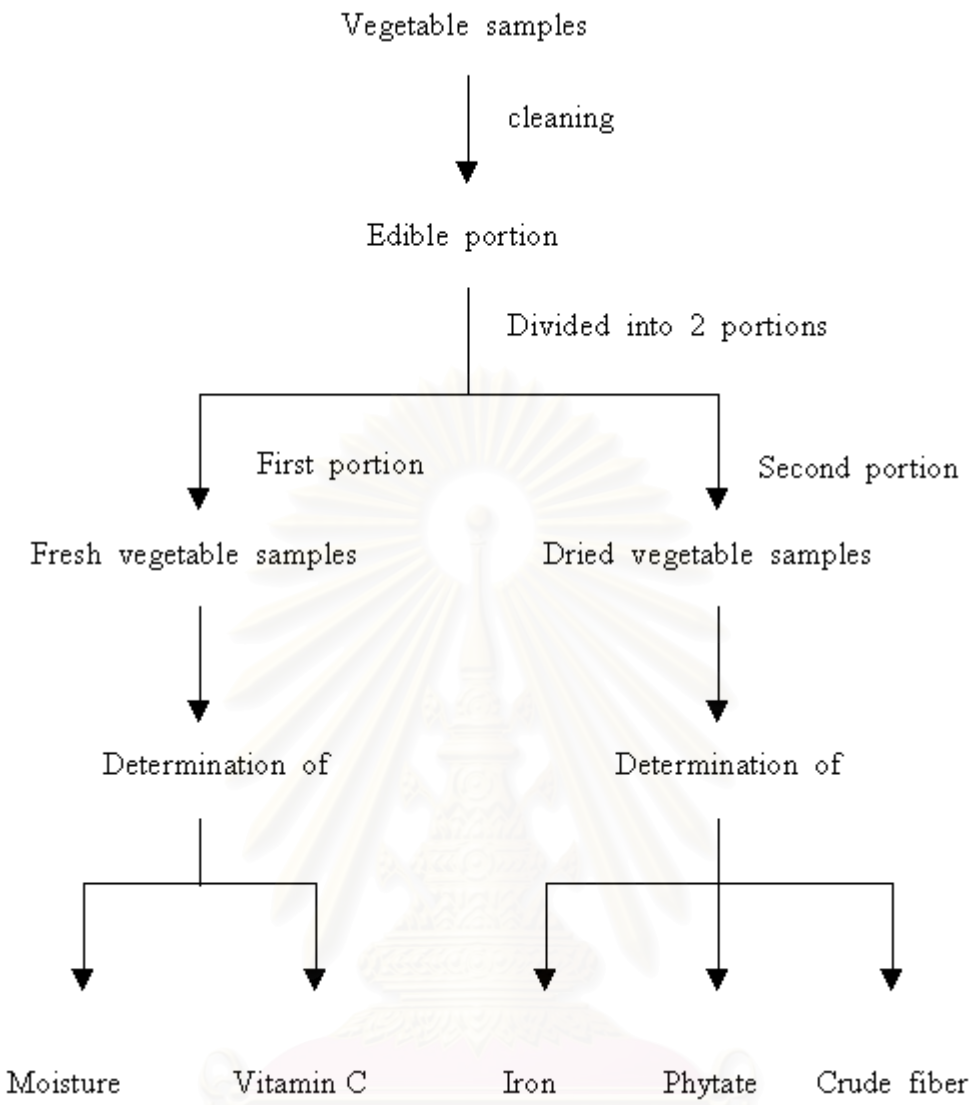


Figure 5. Schematic of experimental design

2. Chemicals

2.1 Sulfuric acid 95-97%, A.R. (E. Merck, Germany)

2.2 Hydrochloric acid 37%, A.R. (E. Merck, Germany)

2.3 Nitric acid 65%, A.R. (E. Merck, Germany)

2.4 Iron standard solution for atomic absorption spectrophotometer, Fe 1.000 mg/ml (Farmitalia Carlo Erba)

2.5 Ascorbic acid, A.R. (Sigma, Germany)

2.6 2,4 – dinitrophenylhydrazine (E. Merck, Germany)

2.7 Metaphosphoric acid, GPR (E. Merck, Germany)

2.8 Thiourea (APS, Australia)

2.9 Activated charcoal (E. Merck, Germany)

2.10 Sodium sulfate (E. Merck, Germany)

2.11 Ferric chloride anhydrous (Sigma, Germany)

2.12 Hydrogen peroxide 30% (E. Merck, Germany)

2.13 Alcohol 95%

2.14 Sodium hydroxide (E. Merck, Germany)

2.15 Petroleum ether, A.R. (Mallinckrodt, U.S.A.)

3. Instruments

3.1 Ultraviolet Spectrophotometer (PYE UNICAM SP 1800)

3.2 Atomic absorption spectrophotometer (Varian Model Spectr AA-300)

3.3 Water bath (Hotech, Model: 905)

3.4 Whatman filter paper no. 1, 4, 541

3.5 Buchner funnel

3.6 Blender (Imarflex super blender IF-308)

3.7 Hot plate (E.G.O., Germany)

3.8Analytical balance (NA 264, Oertling)

3.9 Magnetic stirrer (GEM, Thailand)

3.10 Centriifuge (Funke gerber: Super Vario-N, Germany)

3.11 Extraction unit for determining raw fiber content (VELP Scientifica)

3.12 Glass crucibles (VELP Scientifica)

3.13 Muffle Furnace (Gallenkamp)

3.14 Hot air oven (WTB binder, Germany)

3.15 Desiccator

Method

1. Determination of moisture content (Egan, Kirk and Sawyer, 1981)

About 2 g of each fresh ground vegetable was accurately weighed into a porcelain crucible and dried at 105 °C until its weight became constant. The moisture content of the sample was calculated.

$$\text{Moisture content (\%)} = \frac{\text{sample weight loss x 100}}{\text{initial sample weight}}$$

2. Determination of vitamin C content (Ranganna, 1977)

2.1 About 10 g of fresh vegetable sample was accurately weighed, blended with 50 ml of 10% metaphosphoric acid and made up to 100 ml with 5% metaphosphoric acid and filtered.

2.2 Then 50 ml of solution was transferred in a 100 ml beaker and 1 g of activated charcoal was added. The solution was mixed and filtered through no.1 Whatman filter paper.

2.3 Ten millilitres of the filtrate from 2.2 was pipetted into 100 ml volumetric flask. Five millilitres of 2% thiourea was added and made up to volume with 5% metaphosphoric acid.

2.4 Three test tubes were prepared for each sample. Four millilitres of sample from 2.3 was added into each tube.

2.5 One millilitre of 2% 2,4-dinitrophenylhydrazine solution was added to two test tubes and left one tube without 2% 2,4-dinitrophenylhydrazine solution as blank.

2.6 The tubes were placed in water bath for 3 hours at 37°C. Five millilitres of 85% sulfuric acid was added into tubes and mixed well.

2.7 The tubes were placed in ice bath for 15 minutes. One millilitre of 2% 2,4-dinitrophenylhydrazine solution was added into blank tube and mixed.

2.8 The tubes were removed from ice bath and allowed to stand at room temperature for 30 minutes.

2.9 The absorbance was measured at a wavelength 520 nm. The spectrophotometer was adjusted to read zero absorbance with the reagent reference blank tubes.

Standard curve

About 100 mg of ascorbic acid was accurately weighed and made up to 100 ml with 5% metaphosphoric acid. Then 50 millilitres of solution was transferred in a 100 ml beaker and 1 g of activated charcoal was added. The solution was mixed and filtered through no.1 Whatman filter paper. Ten

millilitres of the filtrate was pipetted into a 250 ml volumetric flask. Five grams of thiourea was added and made up to volume with 5% metaphosphoric acid.

Two, five, ten, fifteen, thirty, fifty and seventy-five millilitres of the above solution was pipetted into separate 100 ml volumetric flasks and made up to volume with 1% thiourea to give a concentration of 0.8, 2, 4, 6, 12, 20 and 30 μg/ml, respectively. After that perform as described above (2.4-2.8) and the absorbance was measured at a wavelength 520 nm. Standard curve was prepared by plotting concentrations against absorbances (Figure 6).

Calculation

The concentrations of ascorbic acid was determined in each sample from the standard curve, and calculated using the following expression.

$$\text{mg / 100 g} = \frac{\mu\text{g as read from the graph} \times \text{Volume made up} \times 100}{\text{ml of sample taken} \times \text{Weight of sample} \times 1000}$$

3. Determination of iron content (Clegg *et al.*, 1981)

Deionized double distilled water was used throughout this study. All glasswares and polyethylene sample containers used in this study were free from iron by being soaked overnight in 10% hydrochloric acid bath and rinsed several times with deionized double distilled water (Osborne and Voogt, 1978).

About 300 – 400 mg of dried and finely ground sample was accurately weighed and transferred to a 50 ml Erlenmeyer flask. Four milliliters of concentrated nitric acid was added to the sample. The flask was kept at room temperature for at least 12 hours for pre–digestion. After that the flask was heated on a hot plate (100 °C) for 4 hours and was allowed to cool. The digestion sample was transferred to a 25 ml volumetric flask and was brought to volume with deionized double distilled water. The digested solution was filtered through a Whatman filter paper no.541 and kept in an airtight polyethylene bottle prior to measure the iron content. Iron content was determined with atomic absorption spectrophotometer.

Standard solutions were prepared from a 1000 ppm iron stock solution at the concentrations of 0.5 , 1.0 , 3.0 and 5.0 ppm in 10% nitric acid solution. The absorbances of standard solutions were determined by atomic absorption spectrophotometer at the following conditions :-

Lamp current	5	mA
Slit width	0.2	nm
Wavelength	248.3	nm
Flame	Air – acetylene	
Background correction	on	
Air flow	13.5	L/min
Acetylene flow	2.0	L/min

Standard curve was prepared by plotting concentrations against absorbances. The concentrations of iron in the digested samples were determined from the standard curve (Figure 7).

4. Determination of phytate content (Thompson and Erdman, 1982)

Deionized double distilled water was used throughout this study. All glasswares and polyethylene sample containers used in this study were free from iron by being soaked overnight in 10% hydrochloric acid bath and rinsed several times with deionized double distilled water (Osborne and Voogt, 1978).

About 2 g of dried and finely ground sample was accurately weighed and transferred to a 250 ml Erlenmeyer flask. One hundred millilitres of 1.2% hydrochloric acid and 10% sodium sulfate were added. The flask was stoppered and shaken for 2 hours on a mechanical shaker. The extract was vacuum filtered through no.4 Whatman filter paper. Ten millilitres of the filtrate was pipetted into a 50 ml centrifuge tube.

Ten millilitres of deionized double distilled water were added, followed by 12 ml of ferric chloride solution (0.2% $FeCl_3.6H_2O$ in 1.2% HCl). The contents were stirred, heated 75 minutes in boiling water bath, cooled and kept for 1 hour at room temperature. The tube was centrifuged at 1130 x g for 20 minutes. The supernatant was decanted and discarded, and the pellet was thoroughly washed two times with 0.6% hydrochloric acid and 2.5% sodium sulfate, respectively. After each wash the contents were centrifuged at 1130 x g for 15 minute and the supernatant was discarded.

Ten millilitres of concentrated nitric acid were added to the resulting pellet and the contents were transferred quantitatively to a 100 ml beaker with several small portions of deionized double distilled water. Four drops of concentrated sulfuric acid were added and the contents were heated approximately 2 hours on a hot plate until only white smoke of the sulfuric acid remained. Approximately 4 ml of 30% hydrogen peroxide were added and the mixture was heated on hot plate at a low heat until bubbling ceased. The resulting solution was made up to 50 ml volume and kept in airtight polyethylene bottle prior to measurement of iron content.

Iron content was determined by atomic absorption spectrophotometer. Phytate phosphorus was calculated from the determined iron assuming a 4 : 6 iron : phosphorus molecular ratio, while phytate was calculated on the assumption that it contains 28.2% of phosphorus.

Standard solutions were prepared from a 1000 ppm iron stock solution at the concentrations of 3.0 , 5.0 , 10.0 and 15.0 ppm in 10% nitric acid solution. The absorbances of standard solutions were determined by atomic absorption spectrophotometer at the conditions perform as described in iron determination.

Standard curve was prepared by plotting concentrations against absorbances. The concentrations of iron was determined from the standard curve (Figure 8).

5. Determination of crude fiber content (Kirk and Sawyer, 1991)

5.1 About 2 g of dried and finely ground sample was accurately weighed and transferred into a 100 ml beaker.

5.2 Thirty millilitres of petroleum ether was added, stirred and decanted three times. The extracted sample was air dried and transferred to a glass crucible.

5.3 Two hundred millilitres of 1.25% sulfuric acid was added in glass steam condenser and boiled 30 minutes exactly from the onset of boiling.

5.4 Sulfuric acid solution was drained by connect to vacuum and washed with hot deionized water until free from acid.

5.5 After drained the last washed, 200 ml of 1.25% sodium hydroxide was added in glass steam condenser and boiled 30 minutes.

5.6 The content was filtered and washed first with 25 ml of hot deionized water then with 25 ml of 1% hydrochloric acid, and with hot deionized water until free from acid and finally with 25 ml of 95% alcohol.

5.7 The glass crucible with residue was removed and this residue was transferred to a porcelain dish.

5.8 The porcelain dish was dried in an oven at 105 °C for an hour and weighed up to constant weight. (= a g)

5.9 Then the porcelain dish and content was ashed in a muffle furnace at 550 °C until all organic matter has been destroyed (approximately 4 hours) and weighed to constant weight. (= b g)

Calculation

$$\% \text{ of crude fiber} = \frac{(a - b) \times 100}{\text{sample weight (g)}}$$

6. Determination of iron bioavailability

The method for calculating the bioavailable dietary iron in each vegetable sample basis on the amount of iron and enhancing and inhibiting factors. Two methods have been proposed for estimating iron bioavailability in this study.

6.1 Monsen's method (Monsen *et al.*, 1978; Monsen and Balintfy, 1982)

Monsen *et al.* (1978) suggested that nonheme iron bioavailability was 3-8%, varying according to the units of enhancing factor (EF), which was calculated as the sum of the milligrams of ascorbic acid and the grams of cooked meat, fish and poultry (MFP) (1.3 g raw MFP was equivalent to 1 g cooked MFP). When EF were not present, only 3% of the nonheme iron would be bioavailable; the nonheme iron bioavailability could reach as high as

8% when EF were greater than or equal to 75 unit. The following two formulas were used:

$$\Sigma EF < 75: \%\ \text{nonheme availability} = 3 + 8.93 \ln [(EF + 100)/100] \quad (1)$$

$$\Sigma EF \geq 75: \%\ \text{nonheme availability} = 8 \quad (2)$$

Therefore, the amount of nonheme iron in each vegetable sample after adjustment for enhancing factor was calculated by the following equation:

nonheme iron bioavailable (mg)

= nonheme iron (mg iron in vegetables) × % nonheme availability

6.2 Tseng's method (Tseng *et al.*, 1997)

Tseng *et al.* (1997) suggested the estimating nonheme iron bioavailability by two steps: first, Monsen's method was used in the calculated absorbable iron; second, the amount of available nonheme iron was adjusted for phytates in the vegetable sample using the following formula:

Log_{10} (% nonheme availability)

= - 0.2869 × $\log_{10}$ (mg phytates in vegetables) + 0.1295.

The formula was derived by fitting data from Hallberg *et al.* (1989) into a logarithmic regression model to estimate parameters.

Therefore, the amount of nonheme iron in each vegetable sample after adjustment for enhancing and inhibiting factors was calculated by the following equation:

nonheme iron bioavailable (mg) = nonheme iron bioavailable (mg) (from Monsen's method) × % nonheme availability (for phytate)

7. Statistical analysis

The contents of iron, vitamin C, phytate and crude fiber were converted to logarithms for statistical analysis and the results were transforms to recover the origin units. In addition, the data were analyzed by simple correlation coefficients between iron, vitamin C, phytate and crude fiber contents of vegetable samples (Gardiner, 1997).

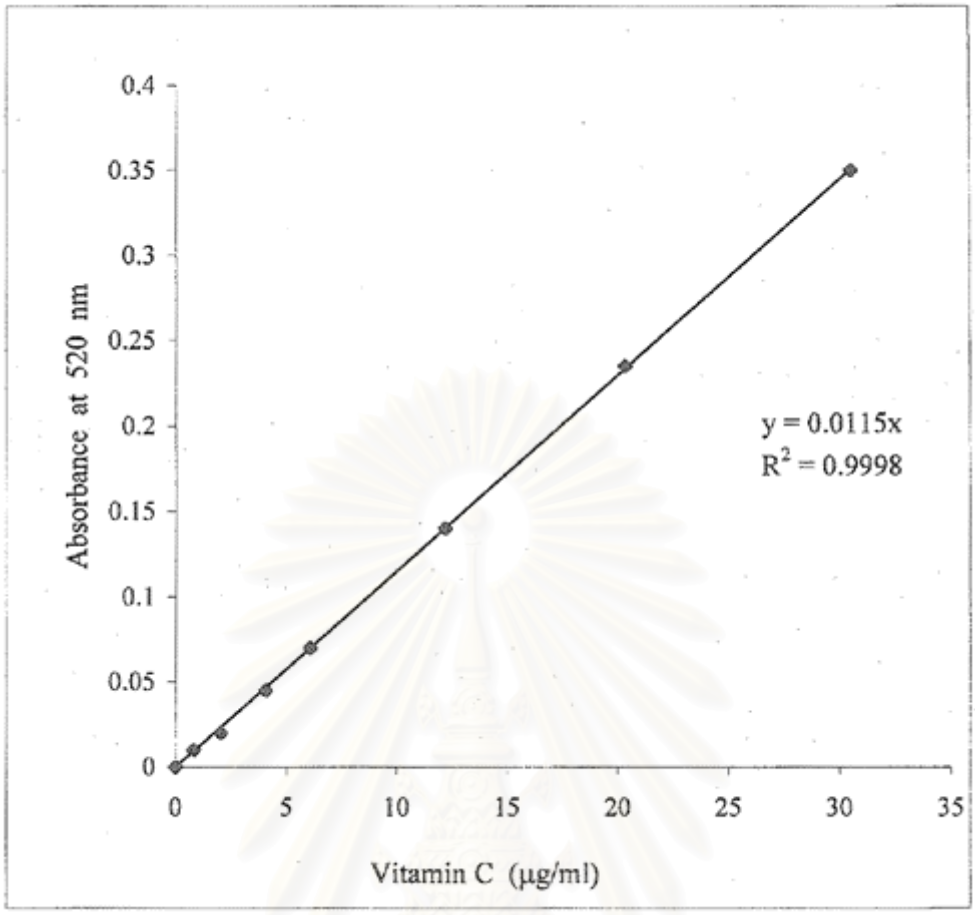


Figure 6. Standard curve of vitamin C concentration vs. absorbance.

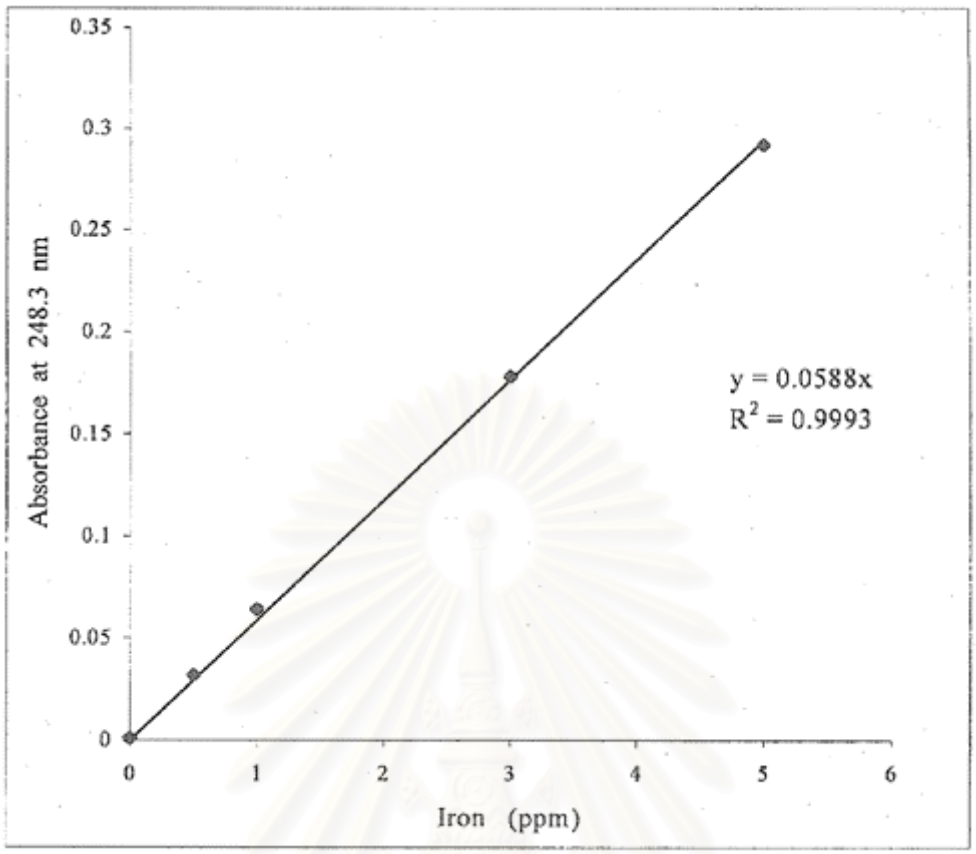


Figure 7. Standard curve of iron concentration vs. absorbance for iron determination.

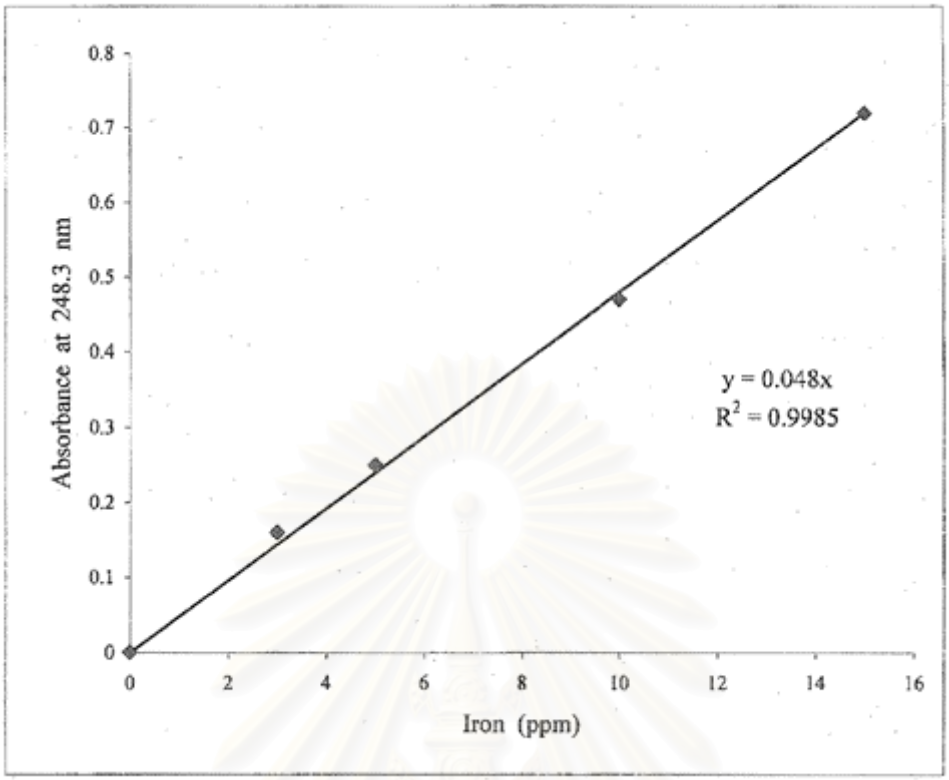


Figure 8. Standard curve of iron concentration vs. absorbance for phytate determination

CHAPTER IV

RESULT

The contents of iron, vitamin C, phytate and crude fiber in the thirty-two studied vegetables are shown in Table 3. The amount of iron, vitamin C, phytate and crude fiber in the studied vegetables was divided into three groups by quatile deviation. The high levels of iron were found in *Spilanthes acmella* Murr (ผักคราด), *Anethum graveolens* Linn (ผักชีลาว), *Polygonum odoratum* Lour (ผักแพว), *Eryngium foetidum* Linn (ผักชีฝรั่ง), *Spirogyra* sp (เทา), *Aponogeton monostachyon* Linn (ผักพาย), *Wolffia globosa* Hartog & Plas (ผักผำ) and *Marsilea crenata* Presl (ผักแว่น) (17.27-62.49 mg/100g dry weight). The moderate amount of iron found ranging from 6.34 mg/100g dry weight in *Luffa acutangula Roxb* (ยอดบวบเหลี่ยม) to 15.11 mg/100g dry weight in *Mordica charantia* Linn (ยอดมะระขี้นก). Other vegetables contained low levels of iron (0.93-6.15 mg/100g dry weight).

The iron absorption enhancer was vitamin C. The high levels of vitamin C were found in *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel (ชะอม), *Spilanthes acmella* Murr (ผักคราด), *Careya sphaerica* Roxb (ผักกระโดน), *Limnophila aromatica* (Lamk.) Merr (ผักแขยง), *Mordica charantia* Linn (ยอดมะระขี้นก), *Anethum graveolens* Linn (ผักชีลาว), *Polygonum odoratum* Lour (ผักแพว) and *Tiliacora triandra* Diels (ใบย่านาง) (46.57-164.68 mg/100g wet weight). The moderate amount

of vitamin C found ranging from 14.61 mg/100g wet weight in *Colocasia gigantea* Hook. f (คูน) to 34.04 mg/100g wet weight in *Allium tuberosum* Roxb (กุยช่าย). Other vegetables contained low levels of vitamin C (8.69-14.57 mg/100g wet weight). Whereas, the iron absorption inhibitors analyzed in this study were phytate and crude fiber.

Among different kinds of vegetables, high levels of phytate were found in *Eugenia grata* Wight. var. *collinsae* Craib (ผักเม็ก), *Eryngium foetidum* Linn (ผักชีฝรั่ง), *Spilanthes acmella* Murr (ผักคราด), *Selaginella argentea* Spriny(พ่อค้าตีเมีย), *Oroxylum indicum* (L.) Vent (ผักลิ้นฟ้า), *Colocasia gigantea* Hook. f (คูน), *Leucaena leucocephala* de Wit (ยอดกระถิน) and *Sesbania grandiflora* (L.) Pers (ยอดแค) (322.83-1184.56 mg/100g dry weight). The moderate amount of phytate found ranging from 128.65 mg/100g dry weight in *Emilia sonchifolia* DC (หูปลาช่อน) to 316.94 mg/100g dry weight in *Allium tuberosum* Roxb (กุยช่าย). Other vegetables contained low levels of phytate (32.66-112.02 mg/100g dry weight).

The high levels of crude fiber were found in *Sesbania grandiflora* (L.) Pers (ยอดแค), *Oroxylum indicum* (L.) Vent (ผักลิ้นฟ้า), *Alpinia nigra* B.L. Burtt (ข่าอ่อน), *Eugenia grata* Wight. var. *collinsae* Craib (ผักเม็ก), *Leucaena leucocephala* de Wit (ยอดกระถิน), *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel (ชะอม) and *Tiliacora triandra* Diels (ใบย่านาง) (2.57-6.56 g/100g dry weight). The moderate amount of crude fiber found ranging from 0.97 g/100g dry weight in

Limnocharis flava (L.) Buchen (ผักคันจอง) to 2.47 g/100g dry weight in *Centella asiatica* (L.) Urban (บัวบก). Other vegetables contained low levels of crude fiber (0.48-0.91 g/100g dry weight).

Table 3. Moisture, Iron, Vitamin C, Phytate and Crude fiber contents in Northeastern local vegetables [a]

Scientific name	Vegetable name		Moisture (%)	Iron (mg/100g)	Vitamin C (mg/100g)	Phytate (mg/100g)	Crude fiber (g/100g)
1. *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel.	Chaom	ชะอม	81.20	9.97	46.57	254.55	4.89
2. *Allium tuberosum* Roxb.	Kui chai	กุยช่าย	91.12	4.94	34.04	316.94	1.14
3. *Alpinia nigra* B.L. Burtt	Kha oon	ข่าอ่อน	91.09	4.96	27.09	224.05	2.78
4. *Amaranthus viridis* Linn.	Pak khom	ผักโขม	89.29	13.35	23.75	244.00	1.15
5. *Anethum graveolens* Linn.	Pak chee lao	ผักชีลาว	91.31	20.56	88.48	41.53	1.09
6. *Aponogeton monostachyon* Linn.	Pak pye	ผักพาย	89.62	35.03	31.34	212.72	1.19
7. *Basella alba* Linn.	Pak prang khao	ผักปลังขาว	91.56	11.24	30.82	254.74	0.87
8. *Basella rubra* Linn.	Pak prang daeng	ผักปลังแดง	92.08	7.28	31.94	179.08	0.90
9. *Careya sphaerica* Roxb.	Pak kradone	ผักกระโดน	82.73	4.17	55.16	75.89	1.35
10. *Centella asiatica* (L.) Urban	Bua bok	บัวบก	88.62	6.79	18.97	242.58	2.47

[a] Each value represents an average

Table 3. Moisture, Iron, Vitamin C, Phytate and Crude fiber contents in Northeastern local vegetables [a] (continued)

Scientific name	Vegetable name		Moisture (%)	Iron (mg/100g)	Vitamin C (mg/100g)	Phytate (mg/100g)	Crude fiber (g/100g)
11. *Colocasia gigantea* Hook. f.	Koon	คูน	92.42	0.93	14.61	850.98	0.78
12. *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin	Pak tew	ผักติ้ว	78.97	5.24	33.62	247.45	2.08
13. *Emilia sonchifolia* DC.	Hoo pla choan	หูปลาช่อน	91.70	7.05	18.94	128.65	1.13
14. *Eryngium foetidum* Linn.	Pak chee farang	ผักชีฝรั่ง	87.87	23.63	19.52	377.03	1.55
15. *Eugenia grata* Wight. var. *collinsae* Craib	Pak meg	ผักเม็ก	85.95	4.48	30.26	322.83	2.96
16. *Ipomoea aquatica* Forsk.	Pak bung thai	ผักบุ้งไทย	92.57	10.17	9.17	63.68	0.87
17. *Lasia spinosa* (L.) Thawait.	Pak nam	ผักหนาม	92.75	7.08	11.39	215.15	0.91
18. *Leucaena leucocephala* de Wit	Yod kratin	ยอดกระถิน	81.58	6.67	10.79	926.81	3.15
19. *Limnocharis flava* (L.) Buchen.	Pak kanjong	ผักคันจอง	93.25	11.69	11.34	100.28	0.97

[a] Each value represents an average

Table 3. Moisture, Iron, Vitamin C, Phytate and Crude fiber contents in Northeastern local vegetables [a] (continued)

Scientific name	Vegetable name		Moisture (%)	Iron (mg/100g)	Vitamin C (mg/100g)	Phytate (mg/100g)	Crude fiber (g/100g)
20. *Limnophila aromatica* (Lamk.) Merr.	Pak khyaeng	ผักแขยง	92.03	12.63	64.86	217.99	1.70
21. *Luffa acutangula* Roxb.	Yod buap liam	ยอดบวบเหลี่ยม	90.30	6.34	11.34	284.91	1.44
22. *Marsilea crenata* Presl.	Pak waen	ผักแว่น	93.35	62.49	23.41	89.43	2.78
23. *Momordica charantia* Linn.	Yod ma ra khi nok	ยอดมะระขี้นก	87.08	15.11	70.96	112.02	1.39
24. *Oenanthe stolonifera* Wall.	Pak chee lom	ผักชีล้อม	90.61	7.74	17.35	148.95	0.88
25. *Oroxylum indicum* (L.) Vent.	Pak linfah	ผักลิ้นฟ้า	84.14	2.61	25.92	819.10	2.65
26. *Polygonum odoratum* Lour.	Pak paew	ผักแพว	75.72	20.73	89.71	178.33	1.81
27. *Selaginella argentea* Spriny. or *Selaginella* spp.	Pho ka tee mia	พ่อค้าตีเมีย	92.06	6.15	11.88	755.32	2.19
28. *Sesbania grandiflora* (L.) Pers.	Yod khae	ยอดแค	80.59	7.97	14.57	1184.56	2.57
29. *Spilanthes acmella* Murr.	Pak khrad	ผักคราด	85.56	17.27	54.52	414.57	2.11

[a] Each value represents an average

Table 3. Moisture, Iron, Vitamin C, Phytate and Crude fiber contents in Northeastern local vegetables [a] (continued)

Scientific name	Vegetable name		Moisture (%)	Iron (mg/100g)	Vitamin C (mg/100g)	Phytate (mg/100g)	Crude fiber (g/100g)
30. *Spirogyra* sp.	Tao	เทา	93.83	28.63	22.15	189.23	0.76
31. *Tiliacora triandra Diels*	Bai ya nang	ใบย่านาง	63.87	7.76	164.68	32.66	6.56
32. *Wolffia globosa* Hartog & Plas	Pak pum	ผักผำ	96.11	45.80	8.69	55.29	0.48

[a] Each value represents an average

The simple correlation coefficients between iron, vitamin C, phytate and crude fiber are shown in Table 4. The positive significant correlation was observed between vitamin C and crude fiber. In contrast, the negative significant correlation was observed between iron and phytate.

Table 4. Simple correlation coefficients between iron, vitamin C, phytate and crude fiber contents in Northeastern local vegetables

	Iron	Vitamin C	Phytate	Crude fiber
Iron	1.000	-0.016	-0.332 **	-0.127
Vitamin C		1.000	-0.236	0.532 *
Phytate			1.000	0.143
Crude fiber				1.000

* Significant at 0.05 level of probability

** Significant at 0.01 level of probability

The amount of absorbable iron in the thirty-two studied vegetables were calculated by the methods proposed by Monsen *et al.* (1978, 1982) and Tseng *et al.* (1997) (Table 5). By Monsen's method, the estimated amount of absorbable iron ranged from 0.04 mg/100g of *Colocasia gigantea* Hook. f (คูน) to 3.05 mg/100g of *Marsilea crenata* Presl (ผักแว่น), the mean value was 0.73 ± 0.68 mg/100g. The amount of absorbable iron calculated by Tseng's method ranged from 0.0076 mg/100g of *Colocasia gigantea* Hook. f (คูน) to 1.13 mg/100g of *Marsilea crenata* Presl (ผักแว่น), the mean value was 0.24 ± 0.26 mg/100g.

Table 5. The amount of calculated absorbable iron of the thirty-two studied vegetables

Scientific name	Vegetable name		Monsen's (1978,1982)[a] mg/100g	Tseng's (1997)[b] mg/100g
1. *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel.	Chaom	ชะอม	0.64	0.17
2. *Allium tuberosum* Roxb.	Kui chai	กุยช่าย	0.28	0.07
3. *Alpinia nigra* B.L. Burtt	Kha oon	ข่าอ่อน	0.25	0.07
4. *Amaranthus viridis* Linn.	Pak khom	ผักโขม	0.65	0.18
5. *Anethum graveolens* Linn.	Pak chee lao	ผักชีลาว	1.64	0.77
6. *Aponogeton monostachyon* Linn.	Pak pye	ผักพาย	1.90	0.55
7. *Basella alba* Linn.	Pak prang khao	ผักปลังขาว	0.61	0.16
8. *Basella rubra* Linn.	Pak prang daeng	ผักปลังแดง	0.40	0.12
9. *Careya sphaerica* Roxb.	Pak kradone	ผักกระโดน	0.29	0.11
10. *Centella asiatica* (L.) Urban	Bua bok	บัวบก	0.31	0.09
11. *Colocasia gigantea* Hook. f.	Koon	คูน	0.04	0.76×10^{-2}
12. *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin	Pak tew	ผักติ้ว	0.29	0.08
13. *Emilia sonchifolia* DC.	Hoo pla choan	หูปลาช่อน	0.32	0.11
14. *Eryngium foetidum* Linn.	Pak chee farang	ผักชีฝรั่ง	1.08	0.27
15. *Eugenia grata* Wight. var. *collinsae* Craib	Pak meg	ผักเม็ก	0.24	0.06
16. *Ipomoea aquatica* Forsk.	Pak bung thai	ผักบุ้งไทย	0.38	0.16
17. *Lasia spinosa* (L.) Thawait.	Pak nam	ผักหนาม	0.28	0.08
18. *Leucaena leucocephala* de Wit	Yod kratin	ยอดกระถิน	0.26	0.05

[a] Iron bioavailability was adjusted for enhancer.

[b] Iron bioavailability was adjusted for both enhancer and inhibitor.

Table 5. The amount of calculated absorbable iron of the thirty-two studied vegetables (continued)

Scientific name	Vegetable name		Monsen's (1978,1982)[a] mg/100g	Tseng's (1997)[b] mg/100g
19. *Limnocharis flava* (L.) Buchen.	Pak kanjong	ผักคันจอง	0.46	0.17
20. *Limnophila aromatica* (Lamk.) Merr.	Pak khyaeng	ผักแขยง	0.94	0.27
21. *Luffa acutangula* Roxb.	Yod buap liam	ยอดบวบเหลี่ยม	0.25	0.07
22. *Marsilea crenata* Presl.	Pak waen	ผักแว่น	3.05	1.13
23. *Momordica charantia* Linn.	Yod ma ra khi nok	ยอดมะระขี้นก	1.18	0.41
24. *Oenanthe stolonifera* Wall.	Pak chee lom	ผักชีล้อม	0.34	0.11
25. *Oroxylum indicum* (L.) Vent.	Pak linfah	ผักลิ้นฟ้า	0.13	0.03
26. *Polygonum odoratum* Lour.	Pak paew	ผักแพว	1.66	0.50
27. *Selaginella argentea* Spriny. or *Selaginella* spp.	Pho ka tee mia	พ่อค้าตีเมีย	0.25	0.05
28. *Sesbania grandiflora* (L.) Pers.	Yod khae	ยอดแค	0.34	0.06
29. *Spilanthes acmella* Murr.	Pak khrad	ผักคราด	1.19	0.29
30. *Spirogyra* sp.	Tao	เทา	1.37	0.41
31. *Tiliacora triandra Diels*	Bai ya nang	ใบย่านาง	0.62	0.31
32. *Wolffia globosa* Hartog & Plas	Pak pum	ผักผำ	1.71	0.73
Mean ± SD			0.73 ± 0.68	0.24 ± 0.26

[a] Iron bioavailability was adjusted for enhancer.

[b] Iron bioavailability was adjusted for both enhancer and inhibitor.

The calculated absorbable iron value of both the Monsen's and Tseng's methods were divided into three groups by quatile deviation (Table 6). The results of Monsen's method showed the high group ranged from 1.18 mg/100g of *Momordica charantia* Linn (ยอดมะระขี้นก) to 3.05 mg/100g of *Marsilea crenata* Presl (ผักแว่น). The medium group ranged from 0.29 mg/100g of *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin (ผักติ้ว) and *Careya sphaerica* Roxb (ผักกระโดน) to 1.08 mg/100g of *Eryngium foetidum* Linn (ผักชีฝรั่ง). The low group ranged from 0.04 mg/100g of *Colocasia gigantea* Hook. f (คูน) to 0.28 mg/100g of *Allium tuberosum* Roxb (กุยช่าย) and *Lasia spinosa* (L.) Thawait (ผักหนาม).

Tseng's method showed the high group ranged from 0.31 mg/100g of *Tiliacora triandra* Diels (ใบย่านาง) to 1.13 mg/100g of *Marsilea crenata* Presl (ผักแว่น). The medium group ranged from 0.08 mg/100g of *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin (ผักติ้ว) and *Lasia spinosa* (L.) Thawait (ผักหนาม) to 0.29 mg/100g of *Spilanthes acmella* Murr (ผักคราด) The low group ranged from 0.0076 mg/100g of *Colocasia gigantea* Hook. f (คูน) to 0.07 mg/100g of *Luffa acutangula* Roxb (ยอดบวบเหลี่ยม), *Alpinia nigra* B.L. Burtt (ข่าอ่อน) and *Allium tuberosum* Roxb (กุยช่าย).

Table 6. The calculated absorbable iron levels of the thirty-two studied vegetables classified by quatile deviation.

Scientific name	Vegetable name		Absorbable iron (mg/100g)	
			Monsen[a]	**Tseng[b]**
High absorbable iron vegetables			**1.17 – 3.05**	**0.31 – 1.13**
1. *Momordica charantia* Linn.	Yod ma ra khi nok	ยอดมะระขี้นก	1.18	0.41
2. *Spilanthes acmella* Murr.	Pak khrad	ผักคราด	1.19	(**)
3. *Spirogyra* sp.	Tao	เทา	1.37	0.41
4. *Anethum graveolens* Linn.	Pak chee lao	ผักชีลาว	1.64	0.77
5. *Polygonum odoratum* Lour.	Pak paew	ผักแพว	1.66	0.50
6. *Wolffia globosa* Hartog & Plas	Pak pum	ผักผำ	1.71	0.73
7. *Aponogeton monostachyon* Linn.	Pak pye	ผักพาย	1.90	0.55
8. *Marsilea crenata* Presl.	Pak waen	ผักแว่น	3.05	1.13
9. *Tiliacora triandra Diels*	Bai ya nang	ใบย่านาง	(**)	0.31
Medium absorbable iron vegetables			**0.29 – 1.16**	**0.08 – 0.30**
1. *Cratoxylum formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin	Pak tew	ผักติ้ว	0.29	0.08
2. *Careya sphaerica* Roxb.	Pak kradone	ผักกระโดน	0.29	0.11
3. *Centella asiatica* (L.) Urban	Bua bok	บัวบก	0.31	0.09

[a] Values estimated using Monsen's method was adjusted for enhancer.

[b] Values estimated using Tseng's method was adjusted for both enhancer and inhibitor.

** The absorbable iron content was classified in medium group

Table 6. The calculated absorbable iron levels of the thirty-two studied vegetables classified by quatile deviation (continued)

Scientific name	Vegetable name		Absorbable iron (mg/100g)	
			Monsen[a]	**Tseng[b]**
Medium absorbable iron vegetables			**0.29 – 1.16**	**0.08 – 0.30**
4. *Emilia sonchifolia* DC.	Hoo pla choan	หูปลาช่อน	0.32	0.11
5. *Sesbania grandiflora* (L.) Pers.	Yod khae	ยอดแค	0.34	(***)
6. *Oenanthe stolonifera* Wall.	Pak chee lom	ผักชีล้อม	0.34	0.11
7. *Ipomoea aquatica* Forsk.	Pak bung thai	ผักบุ้งไทย	0.38	0.16
8. *Basella rubra* Linn.	Pak prang daeng	ผักปลังแดง	0.40	0.12
9. *Limnocharis flava* (L.) Buchen.	Pak kanjong	ผักคันจอง	0.46	0.17
10. *Basella alba* Linn.	Pak prang khao	ผักปลังขาว	0.61	0.16
11. Tiliacora triandra Diels	Bai ya nang	ใบย่านาง	0.62	(*)
12. *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel.	Chaom	ชะอม	0.64	0.17
13. *Amaranthus viridis* Linn.	Pak khom	ผักโขม	0.65	0.18
14. *Limnophila aromatica* (Lamk.) Merr.	Pak khyaeng	ผักแขยง	0.94	0.27
15. *Eryngium foetidum* Linn.	Pak chee farang	ผักชีฝรั่ง	1.08	0.27
16. *Spilanthes acmella* Murr.	Pak khrad	ผักคราด	(*)	0.29
17. *Lasia spinosa* (L.) Thawait.	Pak nam	ผักหนาม	(***)	0.08

[a] Values estimated using Monsen's method was adjusted for enhancer.

[b] Values estimated using Tseng's method was adjusted for both enhancer and inhibitor.

* The absorbable iron content was classified in high group

*** The absorbable iron content was classified in low group

Table 6. The calculated absorbable iron levels of the thirty-two studied vegetables classified by quatile deviation (continued)

Scientific name	Vegetable name		Absorbable iron (mg/100g)	
			Monsen[a]	**Tseng**[b]
Low absorbable iron vegetables			**0.04 – 0.28**	**0.0076– 0.07**
1. *Colocasia gigantea* Hook. f.	Koon	คูน	0.04	0.0076
2. *Oroxylum indicum* (L.) Vent.	Pak linfah	ผักลิ้นฟ้า	0.13	0.03
3. *Eugenia grata* Wight. var. *collinsae* Craib	Pak meg	ผักเม็ก	0.24	0.06
4. *Selaginella argentea* Spriny. or *Selaginella* spp.	Pho ka tee mia	พ่อค้าตีเมีย	0.25	0.05
5. *Luffa acutangula* Roxb.	Yod buap liam	ยอดบวบเหลี่ยม	0.25	0.07
6. *Alpinia nigra* B.L. Burtt	Kha oon	ข่าอ่อน	0.25	0.07
7. *Leucaena leucocephala* de Wit	Yod kratin	ยอดกระถิน	0.26	0.05
8. *Allium tuberosum* Roxb.	Kui chai	กุยช่าย	0.28	0.07
9. *Lasia spinosa* (L.) Thawait.	Pak nam	ผักหนาม	0.28	(**)
10. *Sesbania grandiflora* (L.) Pers.	Yod khae	ยอดแค	(**)	0.06

[a] Values estimated using Monsen's method was adjusted for enhancer.

[b] Values estimated using Tseng's method was adjusted for both enhancer and inhibitor.

** The absorbable iron content was classified in medium group

CHAPTER V

DISCUSSION

The iron content of vegetables consumed by local Northeastern Thai both presented and absence from Thai Food Composition Tables (Puwastien *et al.*, 1999), were determined . The difference of iron content of the same vegetables of Thai Food Composition Table and of this study might be due to the different state of maturity of the analyzed vegetable. Furthermore, the environmental factors of vegetable growing: type of soil, geographic location, climate or seasonal condition during growth, water source and fertilizer used seemed to account for the iron content of vegetable (Lock and Bender, 1980). In addition, the different method of analysis was considerable for the variable value of iron analyzed (Layrisse, Martinez-Torres and Roche, 1968).

Vegetables are probably rich in vitamin C, phytate and crude fiber. Vitamin C is known as the enhancer of iron absorption which presenting in vegetables (Tuntawiroon *et al.*, 1991). The amount of vitamin C in the thirty-two vegetables was in a wide range, 8.69-164.68 mg/100g wet weight. This observation was consistent with the values of Thai Food Composition Table which some substantial difference of vitamin C content among vegetables (Puwastien *et al.*, 1999).

Phytate is known as the inhibitor of iron absorption which presenting in general plants (Hurrel *et al.*, 1992). Boontaveeyuwat *et al.* (1990) categorized phytate content of twenty-nine vegetables as low, medium and high group. The high phytate content was found in young leaf-consumed vegetables which were 'Pak tew', 'pak whan' and Yod khae (562, 519 and 453 mg/100g wet weight, respectively). In this study, the highest phytate content was found in young leaf vegetables, such as in *Leucaena leucocephala* de Wit (ยอดกระถิน) and *Sesbania grandiflora* (L.) Pers (ยอดแค) which contained 926.81 mg/100g dry weight and 1184.56 mg/100g dry weight, respectively.

The distributions of crude fiber content in vegetables were close to each other except *Acacia pennata* (L.) Willd. ssp. *insuavis* Niel (ชะอม) and *Tiliacora triandra* Diels (ใบย่านาง) which were markedly high (4.89 g/100g dry weight and 6.56 g/100g dry weight, respectively).

This study also shows the positive correlation between vitamin C and crude fiber contents ($r = 0.532$ at p-value < 0.05) and negative correlation between iron and phytate contents ($r = -0.332$ at p-value < 0.01). The result demonstrated that if any vegetables contained high amount of vitamin C, the amount of crude fiber would be coincidentally high. On the contrary, if the vegetables contained high amount of iron, phytate would be low. However, There was no correlation between iron, vitamin C and crude fiber contents.

There are several identified dietary factors that influence the absorption of nonheme iron from foods. Most of the data were obtained in human subjects by measuring the absorption of nonheme iron from single meals tagged extrinsically with radioactive iron (Cook *et al.*, 1972). However, there was inadequate information about the absorbable iron in some Thai food items especially vegetables which were the main source of iron for vegetarians and low-income people. The present study determined the absorbable iron from vegetables by calculating method in stead of in vivo method because it could be simple, rapid and low in cost (Hallberg and Hulthen, 2000).

The amount of calculated absorbable iron determined using Monsen's or Tseng's method was based on the amount of iron and vitamin C in vegetables. Moreover, the amount of phytate also was considered in Tseng's method. The mean of absorbable iron of the vegetables from the Monsen's and Tseng's method was 0.73 ± 0.68 mg/100g and 0.24 ± 0.26 mg/100g, respectively. The calculated absorbable iron using Monsen's method was higher than that of Tseng's method because Monsen's method was considered only the effects of enhancing factor (vitamin C); while Tseng's method involved both the effects of enhancing factor (vitamin C) and the effect of inhibiting factor (phytate). Du *et al.* (1999) also found that the mean of calculated absorbable iron of Chinese diet from the Monsen's was higher than Tseng's method about 2 to 3 fold i.e. 1.6 ± 0.9 mg/d and 0.6 ± 0.4 mg/d, respectively.

The calculated values of absorbable iron of vegetables in this study were categorized as low, medium and high absorbable iron by Monsen's and Tseng's method. However, the calculated absorbable iron value of some

vegetable can be categorized in to different groups considered with Monsen's and Tseng's method. The calculated absorbable iron value of *Tiliacora triandra* Diels (ใบย่านาง) obtained with Monsen's method was in a medium range; however, it was in a high range when it was obtained with Tseng's method (Table 6). The difference due to a low phytate content (32.66 mg/100g dry weight) and a high vitamin C content (164.68 mg/100g wet weight). In contrast, *Spilanthes acmella* Murr (ผักคราด) which contained high phytate (414.57 mg/100g dry weight) and high vitamin C (54.52 mg/100g wet weight), obtained with Monsen' method, the absorbable iron was in a high range but when calculated by Tseng' method it was in a medium range.

The inhibition of iron absorption by phytate, present in food, can readily be overcome by a reasonably high dietary intake of vitamin C. Tuntawiroon *et al.* (1990) found that the addition of vitamin C (range: 0, 25 and 50 mg) into low phytate meal (60 mg) increased the percentage of iron absorption from 11.7 to 15.1 and addition of the same amount of vitamin C into high phytate meal (175 mg), the percentage of iron absorption increased from 6.4 to 13.7. Therefore, in diets with a high phytate content, the levels of vitamin C should also be high. The most feasible way to improve iron nutrition in population where the traditional diet contains a high phytate content would probably be to increase the vitamin C content (Hallberg *et al.*, 1989).

Monsen's and Tseng's method had some limitations. First, these methods considered either i) the effects of enhancing factors only or ii) the

effects of both enhancing factors and inhibiting factors, but not simultaneously. Usually many kinds of foods are consumed in a meal and many of them contain both enhancing and inhibiting factors (Siegenberg *et al.*, 1991; Tuntawiroon *et al.*, 1990). Second, these methods ignored other potentially important factors which may affect iron bioavailability such as vitamin A, β-carotene, calcium, fiber, coffee and dietary factors which may affect pH values in the GI tract (Garcia-Cascal *et al.*, 1998; Hallberg *et al.*, 1991; Simpson *et al.*, 1981; Mock, Lynch and Cook, 1983). Estimation of iron availability would be improved by further investigation on the effects and interactions of other dietary factors affecting iron absorption.

If the northeastern inhabitants consumed vegetables as the only source of iron, they can not get enough iron to meet our requirement. In this study, *Marsilea crenata* Presl (ผักแว่น) contained the highest amount of iron (62.49 mg/100g dry weight) when consume this vegetable five servings according to food guide pyramid (Thomas, 1996). The calculated absorbable iron by Monsen's and Tseng's method was 0.35 mg/d and 0.25 mg/d which was lower than the requirement of Thai RDA for adult male and adult female was 10 mg/d and 15 mg/d. On the other hand, *Colocasia gigantea* Hook (คูน) contained the lowest amount of iron (0.93 mg/100g dry weight). The calculated absorbable iron content was 0.0052 mg/d and 0.0019 mg/d by Monsen's and Tseng's method. Therefore, daily consuming should deserve iron from others besides vegetables such as meat, fish and poultry.

The algorithm could then be used to make rough estimates of the bioavailability of diets in some groups of the population with different dietary habits. The algorithm might be useful in the further search for realistic recommendations to be used in food based strategies to improve iron nutrition in the population.

The amount of iron, vitamin C and crude fiber of many foods are presented in food-composition tables while the phytate content is not available and have to be determined in the laboratory, or search from other published data (Harland and Oberleas, 1987; Boontaveeyuwat *et al.*, 1990; Nititham and Srianujata, 1991). This study investigated only vegetables commonly consumed by northeastern Thai. The absorbable iron found would be a guideline for dietary selection in order to decrease the risk of iron deficiency, particularly in the northeastern inhabitants of Thailand.

More studies are needed on the interaction of factors inhibiting and enhancing iron absorption. These may lead to better understanding of the causes of the variation of the bioavailability of iron from different meals and diets, to a better ability to predict the iron absorption from various meals and diets, and to the development of new, effective, and realistic ways to improve iron nutrition in different populations.

CHAPTER VI

CONCLUSION

Thirty-two local vegetables commonly consumed by northeastern inhabitants of Thailand were analyzed for iron, vitamin C, phytate, crude fiber and absorbable iron contents.

The amount of iron ranged from 0.93 mg/100g of *Colocasia gigantea* Hook. f (คูน) to 62.49 mg/100g of *Marsilea crenata* Presl (ผักแว่น).The iron absorption enhancer is vitamin C. The amount of vitamin C ranged from 8.69 mg/100g of *Wolffia globosa* Hartog & Plas (ผักผำ) to 164.68 mg/100g of *Tiliacora triandra* Diels (ใบย่านาง). Whereas, the iron absorption inhibitors analyzed in this study were phytate and crude fiber.

In the present study, the phytate levels ranged from 32.66 mg/100g of *Tiliacora triandra* Diels (ใบย่านาง) to 1184.56 mg/100g of *Sesbania grandiflora* (L.) Pers (ยอดแค). For crude fiber, the lowest was found in *Wolffia globosa* Hartog & Plas (ผักผำ) (0.48 g/100g) while the highest was found in *Tiliacora triandra* Diels (ใบย่านาง) (6.56 g/100g).

The positive significant correlation coefficient was found between vitamin C and crude fiber ($p\text{-value} < 0.05$). In contrast, the negative significant correlation coefficient was found between iron and phytate ($p\text{-value} < 0.01$).

The mean value of calculated absorbable iron of both the Monsen's and Tseng's methods was 0.73 ± 0.68 mg/100g and 0.24 ± 0.26 mg/100g, respectively. The eight high absorbable iron content vegetables were *Momordica charantia* Linn (ยอดมะระขี้นก), *Spirogyra* sp (เทา), *Anethum graveolens* Linn (ผักชีลาว), *Polygonum odoratum* Lour (ผักแพว), *Wolffia globosa* Hartog & Plas (ผักผำ), *Aponogeton monostachyon* Linn (ผักพาย) and *Marsilea crenata* Presl (ผักแว่น). In addition, *Spilanthes acmella* Murr (ผักคราด) was dominated by Monsen's method and *Tiliacora triandra* Diels (ใบย่านาง) by Tseng's method. These vegetables should be encourage to consume especially in the northeastern inhabitants of Thailand.

Recommendation for further study

1. For further study, it is very interesting to calculate absorbable iron in various foods and diet habitually consumed among people. In addition, the effect of cooking and processing on availability of iron in foods should be carry out. Stir-frying and boiling are the main cooking methods of Thai people, which may diminish the effects of phytates and other inhibiting factors as well as ascorbic acid (Svanberg, Lorri and Sandberg, 1993).

2. The iron bioavailability of each vegetable should be further study in the animal and compare with the calculated absorbable iron.

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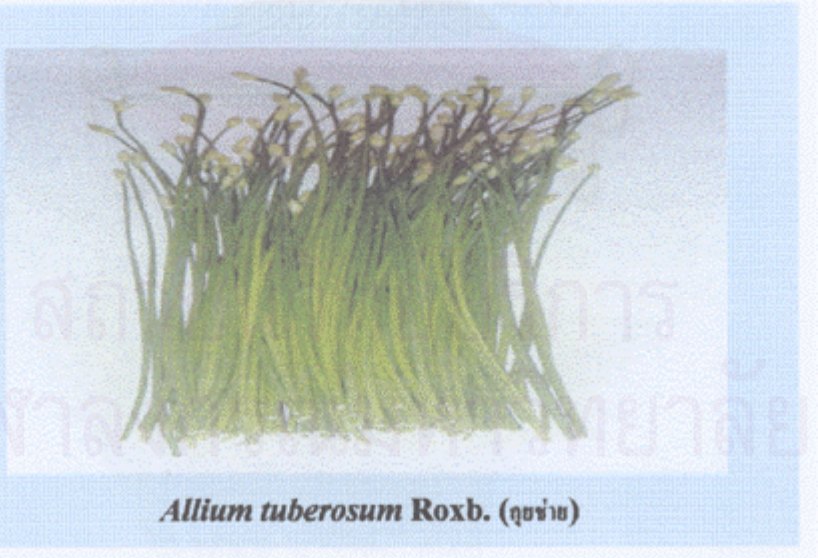
APPENDIX

APPENDIX A

THE PICTURE OF THE THIRTY-TWO STUDIED NORTHEASTERN LOCAL VEGETABLES

Acacia pennata (L.) Willd. ssp. *insuavis* Niel (ขะยอม)



Allium tuberosum Roxb. (กุยช่าย)

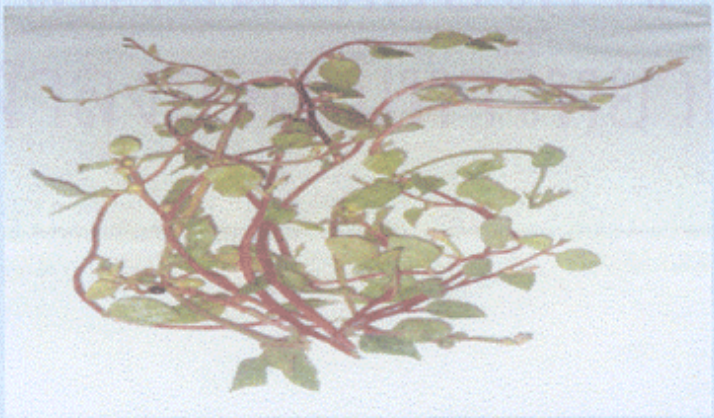
Alpinia nigra B.L. Burtt (ข่าอ่อน)

Amaranthus viridis Linn. (ผักโขม)

Anethum graveolens Linn. (ผักชีลาว)

Aponogeton monostachyon Linn. (ผักพาย)

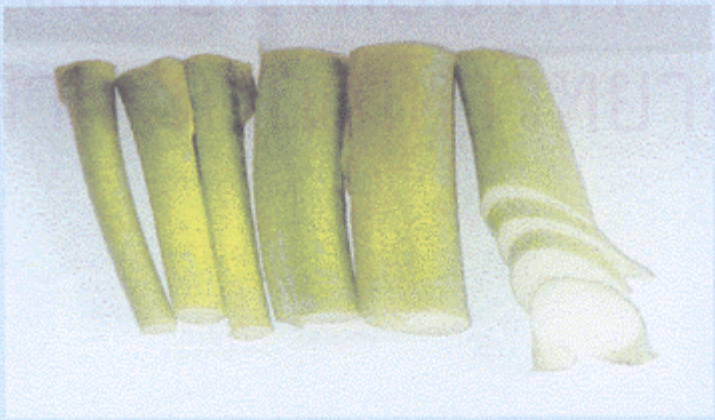
Basella alba Linn. (ผักปลังขาว)



Basella rubra Linn. (ผักปลังแดง)

Careya sphaerica Roxb. (ผักกระโดน)

Centella asiatica (L.) Urban (บัวบก)



Colocasia gigantea Hook. f. (คูน)

Cratoxylum formosum (Jack) Dyer ssp. *pruniflorum* (Kurz) Gogelin (ผักติ้ว)

Emilia sonchifolia DC. (หูปลาช่อน)

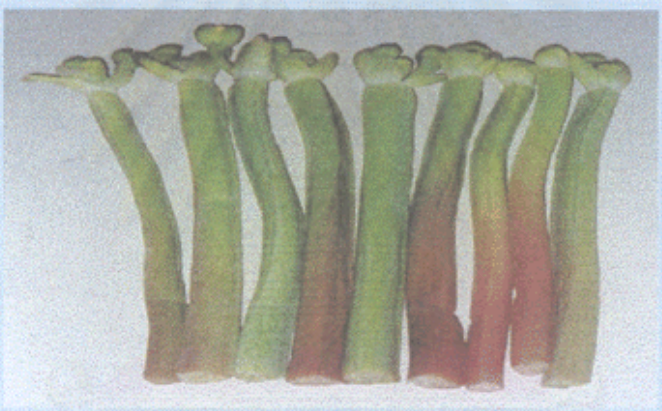
Eryngium foetidum Linn. (ผักชีฝรั่ง)

Eugenia grata Wight. var. *collinsae* Craib (ผักเม็ก)

Ipomoea aquatica Forsk. (ผักบุ้งไทย)

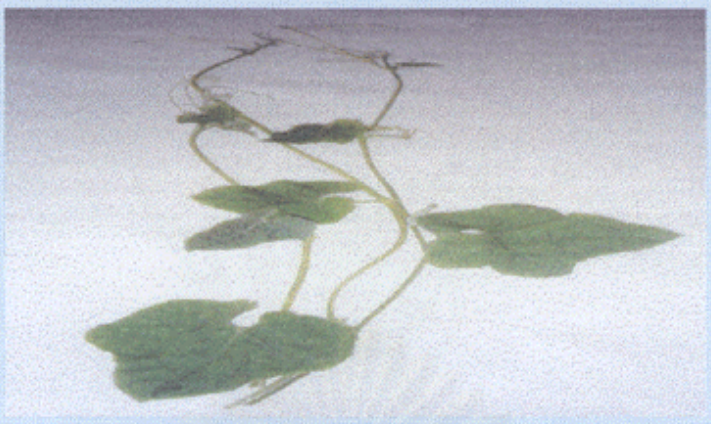
Lasia spinosa (L.) Thawait. (ผักหนาม)

Leucaena leucocephala de Wit (ยอดกระถิน)



Limnocharis flava (L.) Buchen. (ผักคันจอง)

Limnophila aromatica (Lamk.) Merr. (ผักแขยง)

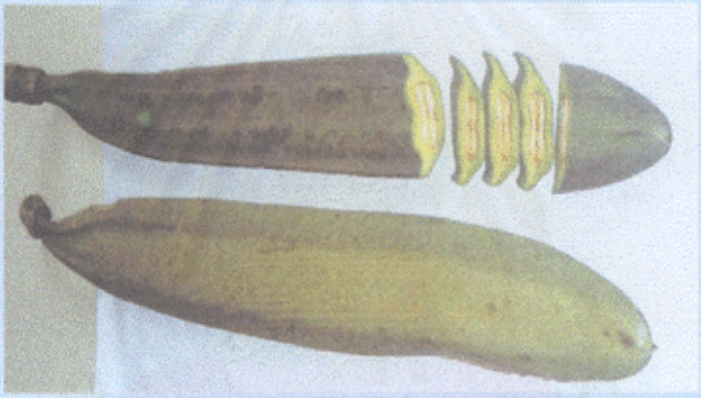


Luffa acutangula **Roxb.** (ยอดบวบเหลี่ยม)

Marsilea crenata **Presl.** (ผักแว่น)

Momordica charantia **Linn.** (ยอดมะระขี้นก)

Oenanthe stolonifera Wall. (ผักชีล้อม)



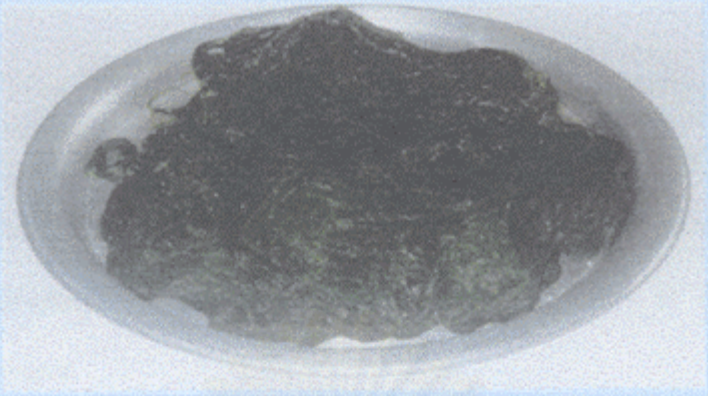
Oroxylum indicum (L.) Vent. (ผักลิ้นฟ้า)

Polygonum odoratum Lour. (ผักแพว)

Selaginella argentea Spriny. (พ่อค้าตีเมีย)

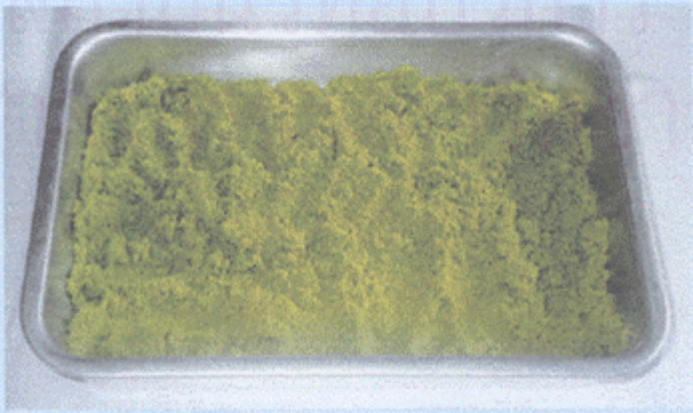
Sesbania grandiflora (L.) Pers. (ยอดแค)

Spilantes acmella Merr. (ผักคราด)



Spirogyra sp. (ตา)

Tiliacora triandra Diels (ใบย่านาง)



Wolffia globosa Hartog & Plas (ผักผ่า)

VITA

Miss Panarat Laohabutr was born on February 1, 1974 in Nakornpathom province, Thailand. She received her Bachelor's degree of science in Pharmacy in 1997 from the faculty of Pharmaceutical Sciences, Khonkaen University, Thailand. After graduation, she works at Phon Hospital, Khonkaen province.