RADIOMETRIC METHOD FOR DETERMINING ABSORPTION OF IRON FROM BREAKFAST MEALS

RUJIRA CHOKCHAI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR
THE DEGREE OF MASTER OF SCIENCE
(RADIOLOGICAL SCIENCE)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
1999

ISBN 974-664-289-8

Thesis

Entitled

RADIOMETRIC METHOD FOR DETERMINING ABSORPTION OF IRON FROM BREAKFAST MEALS

.....
Miss Rujira Chokchai
Candidate

.....
Nopamon Sritongkul, B.Sc.
(Pharm.), M.S.(Biochem.)
Chairman

.....
Malulee Tuntawiroon, B.Sc.,
M.S.(Med.Phys.) M.S.(Nucl.Med.)
Co-advisor

.....
Prof.Liangchai Limlomwongse
Ph.D.
Dean
Faculty of Graduate Studies

.....
Prof.Sutee Na Songkhala, M.D.
Chairman
Master of Science programme
In Radiological Science
Faculty of Medicine,
Siriraj Hospital.

Thesis

entitled

RADIOMETRIC METHOD FOR DETERMINING ABSORPTION OF IRON FROM BREAKFAST MEALS

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Radiological Science)

On
May 30, 2000

.....
Miss. Rujira Chokchai
Candidate

.....
Nopamon Sritongkul, B.Sc.
(Pharm.), M.S.(Biochem.)
Chairman

.....
Aurpun Sutivaree,B.Sc.(Dietetic
& Nutrition),Dip.Appl.Nutri.,
M.A.T. (Home Economics)
Member

.....
Malulee Tuntawiroon,B.Sc.,
M.S.(Med.Phys.),M.S.
(Nucl.Med.)
Member

.....
Prof. Liangchai Limlomwongse Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University

.....
Prof. Chanika Tuchinda
M.D., MS, FAAP.
Dean
Faculty of Medicine,
Siriraj Hospital
Mahidol University

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and deep appreciation to Associate Professor Nopamon Sritongkul and Associate Professor Malulee Tuntawiroon, for their guidance, valuable advice, critics hospitality, and for improve many useful suggestions to this thesis.

I would like to thank Mr.Somchat Maneenoi for his helpful constructive creticisms on statistical analysis in associated with this work; Miss Sunantha Siengjaew for her helpful with photographic work; Mrs.Jumnong Petchkhewna; Mr.Sanae Rujiklin and Mrs.Benja Sawangpetch for their valuable information and assistance during my laboratory study.

I am also thankful to Professor Sutee Na Songkhla, Head of Department of Radiology and Section of Nuclear Medicine for access to laboratory facilities. I am also grateful to Miss Aurpun Sutivaree, Head of the Dietary Department, Siriraj Hospital for being my external examiner.

Finally, I would like to mention an immeasurable support from my parents, Dr. Krittaya Manunapichu M.D.; Dr. Sura Saksilaporn M.D.; Dr. Narongpol Tientong M.D. Head of the Radiology, Maharat Nakhonratchasima hospital and Miss Chanida Phaettut. Without their helps and encouragements this goal would never have reached.

Rujira Chokchai.

4136523SIRS/M : MAJOR : RADIOLOGICAL SCIENCE; M.Sc.(RADIOLOGICAL SCIENCE)
KEY WORDS : IRON ABSORPTION / BIOAVAILABILITY / RADIOMETRIC IN VITRO MEASUREMENT / IRON NUTRITION
RUJIRA CHOKCHAI : RADIOMETRIC METHOD FOR DETERMINING ABSORPTION OF IRON FROM BREAKFAST MEALS. THESIS ADVISORS : NOPAMON SRITONGKUL, M.S., MALULEE TUNTAWIROON, M.S. 143 p. ISBN 974-664-289-8

A study was made on the effect of various drinks and supplement foods on the non-heme food iron availability. The drinks and supplement foods were taken with 3 different groups of breakfast meals. In the first 2 breakfasts, boiled rice and steamed rice meals were added with orange juice, milk, coffee and tea. In the third group, steamed rice meals were added with vanilla flavour cereal, chocolate flavour cereal, milo and ovaltine.

An in vitro radiometric (^{59}Fe) method was used to determine the percentage of food iron ionizability based on the simulation of gastrointestinal digestion and absorption. In the first 2 breakfast meals, orange juice increased the percentage of ionizable iron by 2 to 2.5 times. Milk has no significant effect. A reduction in iron absorption was seen when coffee was added (23 to 60%) or tea was added (85 to 88%) to the meals. In group 3 breakfast a reduction in the percentage of ionizable iron was seen when chocolate flavour cereal was added to the meal (37 to 64%) or milo (54%) or ovaltine (62%) was added. The present study shows that the choice of drink or supplement food to be taken with a meal can markedly affect the availability for non-heme iron.

4136523 SIRS/M : สาขาวิชา : วิทยาศาสตร์รังสี; วท.ม.(วิทยาศาสตร์รังสี)

รุจิรา โชคชัย : การวัดปริมาณธาตุเหล็กที่ถูกดูดซึมจากอาหารเช้า (RADIOMETRIC METHOD FOR DETERMINING ABSORPTION OF IRON FROM BREAKFAST MEALS). คณะกรรมการควบคุมวิทยานิพนธ์ : นกมน ศรีตงกุล M.S., มลุลี ตัณฑวิรุฬห์ M.S. 143 หน้า. ISBN 974-664-289-8

รายงานฉบับนี้เป็นการศึกษาผลของเครื่องดื่ม และอาหารเสริมชนิดต่างๆ ต่อปริมาณธาตุเหล็กในอาหารที่ร่างกายสามารถดูดซึมไปใช้ได้ เมื่อรับประทานร่วมกับอาหารเช้า 3 แบบ ได้แก่ ข้าวต้มและกับข้าวหรือข้าวต้มเครื่อง, ข้าวสวยและกับข้าวแบบง่ายๆรับประทานกับเครื่องดื่มดังนี้ น้ำส้มคั้น, นม, กาแฟและชา ข้าวสวยและกับข้าวหลายชนิด รับประทานกับอาหารเสริมได้แก่ ธัญญพืชรสวานิลลา 2 ชนิด, ธัญญพืชรสชอกโกแล็ต 2 ชนิด, เครื่องดื่มเสริมสุขภาพ ไมโลและโอวัลติน การวัดปริมาณธาตุเหล็กในอาหารที่สามารถดูดซึมได้ ทำได้โดยวิธีเติมสารกัมมันตรังสี (^{59}Fe) ลงในอาหาร, วัดปริมาณธาตุเหล็กในอาหารที่แตกตัวได้เป็นร้อยละ แล้วนำไปคำนวณหาปริมาณเป็นมิลลิกรัมของธาตุเหล็กที่ดูดซึมได้

เมื่อรับประทานน้ำส้มคั้นร่วมกับอาหารเช้า 2 ชนิดแรก น้ำส้มมีผลทำให้ปริมาณธาตุเหล็กในอาหารแตกตัวเพิ่มขึ้น 2 ถึง 2.5 เท่า, นมไม่แสดงผลการเปลี่ยนแปลงอย่างมีนัยสำคัญ กาแฟมีผลทำให้ธาตุเหล็กในอาหารแตกตัวลดลงร้อยละ 23 ถึง 60 ส่วนชาลดลงร้อยละ 85 ถึง 88

ในอาหารเช้ากลุ่มที่ 3 รับประทานร่วมกับอาหารเสริมรสชอกโกแล็ตและเครื่องดื่มรสชอกโกแล็ต มีผลทำให้การแตกตัวของธาตุเหล็กลดลงอย่างชัดเจน ธัญญพืชรสชอกโกแล็ตลดลงร้อยละ 37 ถึง 64 ไมโลและโอวัลติน ลดลงร้อยละ 54 และร้อยละ 62 ตามลำดับ ธัญญพืชรสวานิลลา ชนิดที่ 1 มีผลทำให้ธาตุเหล็กในอาหารแตกตัวเพิ่มขึ้นร้อยละ 27 ในขณะที่อีกชนิดหนึ่งไม่แสดงผลการเปลี่ยนแปลงอย่างมีนัยสำคัญ ผลการศึกษานี้แสดงให้เห็นชัดเจนว่า การเลือกชนิดของเครื่องดื่มหรืออาหารเสริม ที่จะรับประทานร่วมกับอาหารเช้าแบบต่างๆ มีผลต่อปริมาณธาตุเหล็กในอาหารที่ร่างกายสามารถดูดซึมไปใช้ได้

LIST OF CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF CONTENTS	vi
LIST OF TABLES	viii
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiv
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	4
1. Iron	4
2. Body iron compartment	5
3. Iron demands	7
4. Recommended daily iron intake	10
5. Dietary iron	12
6. Iron absorption and its regulation	20
7. Methods for measuring the bioavailability of iron from food	29

LIST OF CONTENTS (Continued)

	Page
CHAPTER	
III MATERIALS AND METHODS	32
IV RESULTS	42
1. General characteristics of test meals	42
2. Determination of food iron ionizability and percentage of the estimated iron availability	50
3. Estimation of iron absorbed from 3 groups of the breakfast meals	56
V DISCUSSION	64
VI CONCLUSION	67
REFERENCES	69
APPENDIX	
A	84
B	92
C	102
D	114
E	121
BIOGRAPHY	127

LIST OF TABLES

TABLE		Page
1	Distribution of iron – containing compounds in the normal human adults.	6
2	Calculated daily iron requirements and recommended daily iron intake as a function of estimated dietary iron bioavailability in various Categories by age and sex.	11
3	Foods and their iron content.	13
4	Relative bioavailability of non – heme iron in a number of foods base largely on single meal studies.	19
5	Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron and iron density in boiled rice meals with different drinks.	44
6	Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron and iron density in simple steamed rice meals with different drinks.	45
7	Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron and iron density in the more complex steamed rice meals with different supplement food.	46
8	Summary of the general characteristics of test meals.	47
9	Summary of the test statistic by Kruskal- Wallis test of the 3 groups of breakfast.	48

LIST OF TABLES (Continued)

TABLE		Page
10	Summary of the test statistic (Mann- Whitney test) for total iron, iron density, total phosphorus and total phytate contents between 2 meals.	49
11	Contents of tannin and catechin in coffee, tea, and chocolate in supplement food.	49
12	The percentage of ionizable iron and estimated iron absorption in boiled rice meals with different drinks.	52
13	The percentage of ionizable iron and estimated iron absorption in simple steamed rice meals with different drinks.	53
14a	The percentage of ionizable iron in the more complex steamed rice meals with different supplement foods.	54
14b	The estimated iron absorption in steamed meals with different supplement foods	55
15	Estimation of the amount of iron expected to be absorbed from boiled rice meals with different drinks.	57
16	Estimation of the amount of iron expected to be absorbed from steamed rice meals with different drinks.	58
17	Estimation of the amount of iron expected to be absorbed from steamed rice meals with supplement foods.	59
18	Discrepancy in p-value of the difference among the means of absorbed iron in 3 different groups of breakfast.	62

LIST OF TABLES (Continued)

TABLE		Page
19	Sample volume for colorimetric analysis.	94
20	Standard solution, 80μg phosphorus/ml.	105
21	Identification and description of the components, inhibitor or promotor in the test meals.	124

LIST OF FIGURES

FIGURE		Page
1	Iron in the diet and a simplified model of the dietary non – heme iron Absorption.	24
2	Mean and median of iron density (mg per 1000kcal) of 3 types of basal Breakfast.	43
3	Distribution of the contents of tannin and catechin (mg/meal) in coffee, tea, milo, ovaltine and chocolate.	50
4	Estimation of the amount of iron expected to be absorbed from boiled rice meals with different drinks.	60
5	Estimation of the amount of iron expected to be absorbed from steamed rice meals with different drinks.	60
6	Estimation of the amount of iron expected to be absorbed from the more complex steamed rice with supplement food.	61
1A	Homogenized food sample.	86
2A	After two-stages (Pepsin-Pancreatin) digestion of homogenized test meal, added chloroform will settle down the lipid from digestion mixture and separate soluble iron in the supernatant.	87
3A	Triplicate of 0.5 ml supernatant of digestion mixture was pipetted into 6 ml screw cap vials.	88

LIST OF FIGURES (Continued)

FIGURE		Page
4A	Bathophenanthroline solution was added to the supernatant of digestion mixture in a screw cap vial. Mix and then shake for 90 minutes at room temperature.	89
5A	The upper isoamyl alcohol was the extracted bathophenanthroline reaction iron from the digestion mixture.	90
6A	Radioactive measurement was counted in the LKB Wallac Gamma counter.	91
1B	Homogenized food sample.	95
2B	Add 2.5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid into the Kjeldahl flask.	96
3B	Digest with low heat and gradually increase the heat until it become dark brown colour.	97
4B	Add 2 ml of 30% hydrogenperxide into the Kjeldahl flask.	98
5B	After added 1 drop of 1% paranitrophenol,slowly add concentrated ammonium hydroxide until the sample turns yellow.	99
6B	After added the chromogen solution becomes red colour and it is directly proportional to the amount of iron in the food sample.	100
7B	The optical density was read in spectrophotometer at 535 nm.	101
1C	Extract the food phytate with 2.4% HCl cover flask and shake at room temperature for 3 hrs.	107

LIST OF FIGURES (Continued)

FIGURE		Page
2C	Prepare the column use glass barrell columns(0.7x15cm) equipped with a valve.	108
3C	Add 0.5 ml concentrated sulfuric acid and 6.0 ml concentrated nitric acid to Kjeldahl flask.	109
4C	Digest under hood on micro-Kjeldahl rack over medium heat.	110
5C	After digested the solution become dark brown colour.	111
6C	The blue colour was developed according to the amount of phosphate after added molybdate-sulfonic acid solution.	112
7C	The optical density was read in spectrophotometer at 640 nm.	113
1D	Prepare tea and coffee in different dilutions.	117
2D	Standard solutions containing tannic acid (TA) and catechin (C).	118
3D	The green colour was developed according to the amount of catechin.	119
4D	The blue colour was developed according to the amount of tannin.	120

LIST OF ABBREVIATIONS

Abbreviations	Term
r.p.m.	revolutions per minute
µl	microlitre
cpm	count per minutes
ml	millilitre
nm	nanometre
µg	microgram
mg	milligram
mCi	millicurie
conc.	concentrate
µCi	microcurie
gm	gram
min.	minutes
kg	kilogram
µgP/ml	microgram of phosphorus per millilitre
µgFe/gm	microgram of iron per gram
RDA	Recommended Dietary Allowances
mgFe	milligram of iron
MW	Molecule Weight
M	Mole
WHO	World Health Organization

LIST OF ABBREVIATIONS (Continued)

Abbreviations	Term
FAO	Food and Agriculture Organization
kcal	kilocalorie
STD	Standard
hrs.	hours
sec.	second

CHAPTER I

INTRODUCTION

Iron is an essential trace element which plays a role of central importance in oxygen transport by haemoglobin and myoglobin in human body. It involves in many reaction of the cellular metabolism, notably in mitochondrial electron transport, the citric acid cycle, detoxification reaction and the synthesis of deoxyribonucleotides (1,2). Iron in excess of functional needs is stored within the body as ferritin and haemosiderin, which are specially designed for holding it in a relatively non- reactive form. Altogether man has a complement of 35 to 40 mg per kg of functional iron and a reserve of between 0 and 20 mg per kg of storage iron (3).

The iron in food is absorbed in order to maintain the supply of iron to all cells.There are two kinds of iron in the diet with respect to mechanisms of absorption: heme and non- heme iron. Heme iron is derived mainly from meat which is generally high biovailability, its absorption is not affected by the composition of the diet. Non-heme iron in rice, vegetables, cereals, fruits forms the main part of dietary iron. The absorption is very much influenced by a number of dietary factors (4,5,6,7). Although heme iron is well absorbed, meat has virtually disappeared from the diet of many people, particularly in developing countries. It is the iron in most vegetable staples, which is usually of low availability and is considered to be one of the most

factors in the etiology of iron deficiency. Iron deficiency result when the amount of iron absorbed from the diet cannot meet physiological requirement – the basal losses of body iron, the menstrual iron losses, the requirements for pregnancy and for growth in infants and children. By using an extrinsic tag method (8,9,10) inorganic tracer uniformly labels the non- heme iron in a meal, made it possible to measure food iron absorption from different types of meals and to study various factors influencing absorption. The absorption of non- heme iron is variable, being dependent on the relative proportions of promotors and inhibitors of iron absorption present in the diet. It is important to obtain more information about factors affecting the bioavailability of dietary iron in Thai meals. While the most reliable method for determining availability of iron from various food sources is to measured iron absorption in human using extrinsic tag method, it is time consuming and unsuited to the screening of large numbers of meals or food items. Several attempts have been made to design in vitro systems that will accurately predict absorption in human (11,12,13). In the systems determining of ionizable, soluble or dialyzable iron after enzymatic digestion under physiological condition have been developed (14,15). A variety of in vitro methods have been shown to have advantages for rapid screening large number of foods and diets for predicting the iron availability from them and to evaluate dietary factors that influence absorbed iron requirements (16,17). Since no work have been done on food iron ionizability from common Thai breakfast. The determination of ionizable iron by an in vitro method is interested for investigation . In this thesis we have described a modified in vitro procedure for predicting dietary iron availability based on iron ionizability determination in HCl- pepsin followed by incubation with pancreatic

enzyme and pH adjustments to level close to neutrality to simulate gastrointestinal digestion of food iron.

The purpose of this thesis is to determine whether this modified method can be applied in evaluating a more precise estimation of iron absorption and provide the information on iron nutrition of the common Thai breakfast. To study the effect of different drinks (orange juice, milk, coffee and tea) and supplement food (nutritions cereal, milo, ovaltine) on the non- heme iron ionizability which were served with breakfast. This study may lead to a better understanding of a drink taken together with a meal which may affect the availability of dietary iron by its content of factors that enhance or reduce the ionizability of iron. The results could be useful to improve iron nutrition by diet planning for higher dietary iron intake and choocing of drink to be taken with meal for promoting iron availability or between meal because of its inhibiting effect when served with the meal.

CHAPTER II

LITERATURE REVIEW

1. Iron

Iron is an essential transition metal with and atomic weight of 55.85. Although it presents in living organisms in only minute amounts but participates in a number of biologic processes essential to life. It exists not in the free state but as part of several compounds necessary for oxygen transport and oxidative processes. The major portion of body iron is found as the iron porphyrin complexes, hemoglobin, myoglobin and a variety of haem-containing enzymes. About 60 to 70 percent of the 3 to 5 g total body iron is present in hemoglobin, some 3 to 5 percent in muscle myoglobin and about 10 percent in other cellular proteins (18). The remaining iron is stored within the body as ferritin and haemosiderin. The distribution of iron-containing compounds in the human body is given in Table 1. Iron is required by the body in quantities of few milligrams per day and absorbed from food. Food iron is classified as heme and non-heme iron. Heme is derived from meat and forms an important source of dietary iron intake because of its high bioavailability. All iron in plants are non-heme, various dietary factors markedly affect its absorption, where as the absorption of heme iron is much less affected by the other ingredients of the meal.

The iron requirements varied widely in different individuals depending on their age and sex.

2. Body iron compartments

Two major compartments, namely functional and storage iron can be considered for body iron (3). Functional iron accounting for 35 - 40 mg per kg is in the form of erythrocyte hemoglobin, smaller amount in muscle myoglobin, together with a number of heme and non-heme iron containing enzymes which are essential for cellular metabolism, growth and division (19,20). The storage iron compartment is an intrinsically more variable compartment accounting in normal individuals for between 0 and 20 mg iron / kg body weight (21). The iron in this compartment is stored in association with proteins in non-reactive form in the compounds ferritin and hemosiderin. It is located primarily in the reticuloendothelial system that consists of macrophage elements found predominantly in the bone marrow, liver and spleen, and in parenchyma cells of the liver. It serves both as a repository for dietary iron absorbed in excess of functional requirements and as an emergency reserve supply for sudden deficits in functional iron requirements. The interfacing system between storage and functional compartments is transport system that may be considered as a third compartment. In this compartment iron is bound to transferrin. The transferrin has been traditionally characterized as a class of two-sited iron binding protein which carry iron to meet tissues need throughout the body. This small fraction of transport iron is about 0.2 mg per kg. It is distributed throughout most of the extracellular fluid

(22) of the body, with a continuous circulation from plasma to interstitial fluid and then back to the blood via the lymph vessels.

TABLE 1. Distribution of iron – containing compounds in the normal human adults (18).

Compound	Total in Body (g)	Iron content (g)	Percent of total iron in body
Iron porphyrins (heme compounds)			
Hemoglobin	900	3.0	60 – 70
Myoglobin	40	0.13	3 – 5
Heme enzymes			
Cytochrome c	0.8	0.004	0.1
Catalase	5.0	0.004	0.1
Other cytochromes	-	-	-
Peroxidase	-	-	-
Nonporphyrin iron compounds			
Siderophilin (transferrin)	10	0.004	0.1
Ferritin	2.4		30
Hemosiderin	1.6	1.2 – 1.5	
Total iron		4.0 - 5.0	100

3. Iron demands

Iron must be obtained from the diet in order to replace obligatory losses from the body, and, to provide for growth not only from infant to childhood but also childhood to adulthood.

3.1 Basal Iron Losses

The total amount of basal iron lost in adult males is about 12 to 14 μg/kg/day (23,24). It is passively lost from the desquamation of surface cells from the skin, epithelial cells from lumen of the gastrointestinal and urinary tracts, and small amounts of gastrointestinal blood loss. In females because of smaller body surface areas, the losses of iron would be expected to be correspondingly less and can be assumed to be in the range of 0.7 - 0.8 mg daily compared to 0.9-1.0 mg daily in males.

3.2 Increases Iron Losses

3.2.1 Menstruation

Monthly menstrual blood loss in the adult female is between 20 and 30 mg. It is highly variable between women and constant within any individual (25,26). This loss increases daily requirements from 0.8 mg to 1.47 mg and up to 2.84 mg in 95 percent of females to maintain iron balance (27). Variation in menstrual losses may become from the methods of contraception. With oral contraceptives reduces losses by approximately 50 percent (28) and intra-uterine devices which doubles menstrual iron losses (29).

3.2.2 Pregnancy

In pregnancy the first trimester iron needs one actually lower than in the nonpregnant state, due to the cessation of menstrual iron losses. The requirements increase during the second and third trimester for the exponential growth of placenta and fetus, the linear growth of red cell mass and basal iron losses by the mother (30). The amount of iron needs in 9 – months gestation for a 55 kg woman are as follows : 360 mg for the products of conception (fetus 270 mg, placenta and umbilical cord 90 mg), 230 mg for basal, 150 mg as peripartum blood loss. Total iron loss in pregnancy is 740 mg. An additional 450 mg of iron required for expanded red cell mass is not included because of temporary need for total oxygen transport to mother and fetus, it is returned to the mother during postpartum contraction of red cell mass (31).

3.2.3 Pathological blood loss

It was found that the main cause of pathological blood loss is in the gastrointestinal tract. Oesophagitis, gastritis, peptic ulcers, neoplasm, liver cirrhosis, inflammatory bowel disease and hemorrhoids are the example of gastrointestinal blood loss (32,33). Moreover the parasitic infestation, particularly by hookworm is the secondary pathological blood loss. In heavy parasitization with faecal egg loads in the region of 5000 per gm may increase daily iron requirements by as much as 3 – 4 mg (34).

3.2.4 Non – pathological blood loss

The widespread use of aspirin for its cardio – protective and anti - inflammatory properties, blood donors and autologous blood banking prior to elective surgery are the example of some situations in which blood loss increases as a

consequence of neither pathology nor normal physiology. Regularly used of aspirin has been well - documented to enhance intestinal loss of red blood cells which can be as much as ten times the basal loss (35,36). Other therapeutic agents such as anticoagulants, corticosteroids, non-steroidal anti-inflammatory agent have the same effects. Blood donation increases iron losses, with one donation of 500 ml blood per year imposes and additional daily iron requirement of 0.5 mg (37).

3.3. Growth

Iron requirements are increases for growth and expansion of erythropoiesis in infants, children and adolescents.

3.3.1 Infancy

Total body iron content of normal full - term infant is 80 mg per kg (38) about one - third is in storage form (39) and two-thirds in circulating haemoglobin (40). A newborn infant weighing 2 kg would have about 160 mg of iron, enough to support growth to about 4 kg, while a 4 kg infant with 320 mg would have sufficient iron to support growth to 8 kg. Rapid growth in infant increases requirements, however it is independent of nutritional iron because of the low intake, so the stores are depleted. Growth and subsequent erythropoiesis exhausts the stores within six months. The body iron content at birth is directly proportional to the birth weight and defines the amount of iron that must be obtained from the diet during infancy. The full - term infant who has started life with a full complement of about 270 mg iron need absorb only about 100 mg during the whole year or 0.3 mg per day. For premature or low birth weight infants may require up to 1 mg per day (41).

3.3.2 Childhood

Iron requirement for the growth rate during the second year of life is approximately 0.4 mg per day. From the second birthday to the twelfth in the male and the tenth in the female, the mean increase in body weight is about 2.5 – 2.75 kg per year, equivalent to an iron requirement of about 0.3 mg per day. It is less than that of infancy on account of the reduced growth rate from two years to the beginning of adolescence. A rise in the haemoglobin concentration and an increase in obligatory losses during this period from 0.5 to 0.8 mg further increase the daily requirement.

3.3.3 Adolescence

Adolescence is the period between childhood and adulthood. It is a time of rapid growth and physical and psychological development. The growth spurt of adolescence accelerates the mean increase in body mass to about 4.6 kg per year in the male and 4 kg per year in the female. The daily iron requirement for growth alone in male and female is about 0.7 mg and 0.45 mg, respectively. Adolescence females also have an additional demand for menstruation. The overall daily iron requirements for adolescents are estimated to be 1.45 – 2.03 mg in boys and 1.22 – 1.46 mg in pre-pubertal girls and 1.39 – 2.54 mg after menstruation has started (42).

4. Recommended daily iron intake

Daily iron requirements are widely individuals different depending upon their age and sex, women of fertile age having the greatest need of iron. The smallest amount of 18 μg/kg/day is an adequate iron requirement for the post-menopausal

women and adult men. In pregnancy during second and third trimester requirements cannot be met by diet alone. The estimated iron requirements and recommended daily iron intakes for the various groups stratified by age and sex at 95th percentile of the population, which based on the recent report of the FAO/WHO Expert Consultative Group (43) are summarized in Table 2.

TABLE 2. Calculated daily iron requirements and recommended daily iron intake as a function of estimated dietary iron bioavailability in various categories by age and sex. Values are based on 95th percentile calculations.

Group	Age (yrs)	Requirements (μg/kg/day)	Recommended intake (mg/day)		
			Low (5%)	Intermediate (10%)	High (15%)
Children	0.25 – 1	120	21	11	7
	1 – 2	56	12	6	4
	2 – 6	44	14	7	5
	6 – 12	40	23	12	8
Boys	12-16	34	36	18	12
Girls	12 – 16	40	40	20	13
Adult men		18	23	11	8
Adult women :					
Menstruation*		43	48	24	16
Post-menopausal		18	19	9	6
Lactating		24	26	13	9

*Recommended intakes for Low Intermediate bioavailability are unlikely to be achieved with conventional diet.

5. Dietary iron

Dietary ingredients and products of digestion exert a profound influence on the availability for absorption of the ingested iron, and the amount that can be absorbed is determined by not only the iron content of the diet but also its exact composition. Therefore the absolute iron content and the bioavailability of iron are the two essential components in the ingested food which influence the role of dietary iron in iron balance.

5.1 Food iron content

The iron content of foods varies widely, which leads to great differences in the supply of iron through different diets. Even the same food may show extensive variations in iron content depending upon its origin, habitat and treatment. Large variations in iron content have been found in vegetable foods even when grown under normal condition (44). With chemical analysis of typical Western meals indicate that the iron content is generally about 6 mg / 1000 kcal (45,46). Examples of the iron content of some of the most common foods are given in Table 3. From the table milk and most milk products contain only from 0.06 to 0.58 mg/100 g. In many fruits and berries such as apples, bananas, oranges, cantaloupe, pears, watermelon and grapefruit, the iron content is less than 1 mg / 100 g. Meat, fish and whole eggs have the moderate iron content of 1 – 5 mg / 100 g. Many vegetables (spinach, mustard greens, turnip greens and beet greens) have and average iron content of 3 mg / 100 g. Liver, egg yolk and oysters are classified into high iron content foods with the iron content more than 5 mg per 100 g. Together with dried beans and wheat - germ have a relatively high iron content of about 8 mg per 100 g.

TABLE 3. Foods and their iron content (47).

Foods of low iron content (0-0.99mg/100 g)	mg/100 g	Foods of moderate iron content (1.0-4.99mg/100 g)	mg /100g	Foods of high iron content (5.0-18.2mg/100 g)	mg/100g
Buttermilk	0.07	Mayonaise	1.0	Egg yolk, fresh	7.2
Chocolate milk	0.07	Eggs, whole, fresh	2.7	Eggs whole, dried*	8.7
Dry skimmed milk	0.58	Chuck roast	2.8	Corned beef, dried or chipped*	8.1
Dry whole milk	0.58	Corned beef, canned	4.0	Liver, fresh	12.1
Evaporated milk	0.17			Heart, fresh	6.2
Fresh skimmed	0.07	Hamburger	2.4	Liver sausage	5.4
Fresh whole milk	0.07	Loin steaks	2.5	Tongue, fresh	6.9
Cream	0.06	Round steaks	2.9	Oysters	7.1
Ice cream	0.10	Stewing meat	2.4	Beans, dry – seed, Common or kidney*	10.3
Cheddar cheese	0.57	Lamb	2.7	Beans,lima,dry-seed *	7.5
Cottage cheese	0.46	Ham	2.3	Peas, spread *	6.0
Cream cheese	0.17	Pork chop	2.5	Soy-beans, whole, mature	8.0
Bacon	0.8	Pork link sausage	1.6	Peaches,dried (sul-phered)*	6.9
Butter	0.2	Veal	2.9	Oatmeal*	5.2
French dressing	0.1	Baloney	2.2	Bouillon cubes*	9.2

TABLE 3. (Continued) Foods and their iron content (47).

Foods of low iron content (0-0.99mg/100 g)	mg/100 g	Foods of moderate iron content (1.0-4.99mg/100 g)	mg/100g	Foods of high iron content (5.0-18.2mg/10 g)	mg/100g
Lard & other Shortening	0	Frankfurter	2.3	Wheat – germ	8.1
Oleomargarine	0.2	Hash	1.3	Yeast, brewers	18.2
Salad & cooking oil	0	Chicken	1.9		
Cod	0.9	Turkey	3.8		
Asparagus, fresh	0.9	Salmon	1.3		
Cabbage, fresh	0.5	Sardines	1.8		
Carrots, fresh	0.8	Shrimp	2.0		
Celery, fresh	0.5	Tuna	1.7		
Corn, fresh	0.5	Beans, canned	3.4		
Cucumbers, fresh	0.3	Almonds	4.4		
Eggplant, fresh	0.4	Peanut butter	1.9		
Lettuce	0.5	Peanuts, roasted	1.9		
Okra	0.7	Pecans	2.4		
Onions, mature	0.5	Walnuts, english	2.1		
Parsnip	0.7	Beans lima, green	2.3		
Peppers, green, fresh	0.4	Beet greens	3.2		
Potatoes, white	0.7	Beets	1.0		
Rutabegas, fresh	0.4	Broccoli	1.3		
Squash, fresh	0.4	Brussel sprouts	1.3		

TABLE 3. (Continued) Foods and their iron content (47).

Foods of low iron content (0-0.99mg/100 g)	mg/100 g	Foods of moderate iron content (1.0-4.99mg/100 g)	mg /100g	Foods of high iron content (5.0-18.2 mg /100 g)	mg/100g
Squash, winter	0.6	Cauliflower	1.1		
Sweet potatoes	0.7	Chard	4.0		
Tomatoes	0.6	Dandelion greens	3.1		
Turnips	0.5	Kale	2.2		
Canned corn	0.5	Lettuce	1.1		
Canned Sauerkraut	0.5	Mustard greens	2.9		
Tomato catsup	0.8	Peas, green	1.9		
Tomato juice	0.4	Radishes	1.0		
Tomatoes canned	0.6	Spinach	3.0		
Apples, fresh	0.3	Lima beans	1.7		
Apricots, fresh	0.5	Peas, green, canned	1.8		
Avocado	0.6	Spinach, canned	1.6		
Banana	0.6	Tomato puree	1.1		
Blueberries	0.8	Beets, puree	1.8		
Strawberries	0.8	Beans, green, puree	1.0		
Other berries	0.9	Carrots, puree	1.0		
Cantaloupe	0.4	Greens, mixed	1.4		
Grapefruit	0.3	Peas, puree	1.8		
Grapes	0.6	Spinach, puree	1.0		
Lemon	0.1	Vegetable soup	1.0		
Orange	0.4	Plums, canned	1.1		

Table 3. (Continued) Foods and their iron content (47).

Foods of low iron content (0-0.99mg/100g)	mg/100 g	Foods of moderate iron content (1.0-4.99mg/100 g)	mg /100g	Foods of high iron content (5.0-18.2mg/100 g)	mg/100g
Peach	0.6	Apricots, dried	4.9		
Pear	0.3	Prune, dried	3.9		
Pineapple	0.3	Fruits (apricots & apples, strained)	1.3		
Plums	0.5	Pears & pineapple, strained	1.3		
Tangerines	0.4	Cornmeal,whole-grain	2.7		
Watermelon	0.2	Wheat flour, enriched	2.9		
Applesauce, canned	0.2	Whole-wheat flour	3.8		
Apricots, canned	0.3	Whole - wheat bread	2.6		
Cherries, canned	0.3	Crackers	1.5		
Cranberry sauce	0.3	Cornflakes	1.0		
Orange juice	0.4	Wheat flakes, puffed	3.7		
Pineapple juice	0.5	Shredded wheat	3.8		
Pears, canned	0.2	Barley, pearled	2.0		
Pineapple,canne	0.6	Hominy	1.0		

TABLE 3. (Continued) Foods and their iron content (47).

Foods of low iron content (0-0.99mg/100 g)	mg/100 g	Foods of moderate iron content (1.0-4.99mg/100 g)	mg /100g	Foods of high iron content (5.0-18.2mg/100 g)	mg/100g
Cornstarch	0	Macaroni,spaghetti	1.2		
Rye bread	0.8	Noodles	1.9		
Rice, puffed or flakes	0.9	Tapioca	1.0		
Farina	0.8	Sugar, brown, dark	2.6		
Rice, white	0.7	Syrub, table blend	4.1		
Honey	0.9	Cocoa	2.7		
Jam, jellies	0.3	Coconut, dried	3.6		
Sugar, granulated	0.1				
Gelatin dessert powder	0				
Wine	0.3				

* It should be note that the iron content in these foods is based on dry weight and that this value will be variably reduced upon preparing the food.

5.2 Food iron bioavailability

While the dietary iron intake is obviously important, of even greater nutritional significance is the bioavailability of food iron (48). The bioavailability of dietary iron can be classified into two forms : heme iron, which is easily absorbed whatever the composition of the diet, and non- heme iron, which is usually of low bioavailability. Non - heme iron is the largest component whatever of its origin in a meal, it has been shown to behave to a large extent as a single pool and its absorption is influenced by the balance between promotors and inhibitors which may be present. The availability of food iron in a Western diet is in the range of 14 – 17 percent (27). As the iron content expressed in relation to food caloric value of 6 mg/1000 kcal a 2000 kcal food intake could provide 1.8 mg iron per day. This is insufficient to meet the requirements of many menstruating women and all pregnant women. Excess of a 2000 kcal diet must be ingested to obtain sufficient iron. For US women would be 3264 kcal and 3440 kcal for menstruating females in Sweden (27). Using the single meal studies it is possible to arrange foods in terms of relative bioavailability of the non-heme iron as shown in Table 4 (49).

TABLE 4. Relative bioavailability of non - heme iron in a number of foods based largely on single meal studies (49) .

Foods	Bioavailability		
	Low	Medium	High
Cereals	Maize	Cornflour	
	Oatmeal	White flour	
	Rice		
	Sorghum		
	Whole wheat flour		
Fruits	Apple	Cantaloupe	Guava
	Avocado	Mango	Lemon
	Banana	Pineapple	Orange
	Grape		Papaya
	Pear		Tomato
	Peach		
Vegetables	Legumes	Potato	Broccoli
	Soy flour		Cabbage
	Isolated soy protein		Cauliflower
	Lupines		Pumpkin
			Turnip
Beverages	Tea	Red wine	White wine
	Coffee		

TABLE 4. (Continued) Relative bioavailability of non - heme iron in a number of foods based largely on single meal studies (49).

Foods	Bioavailability		
	Low	Medium	High
Nuts	Almond		
	Brazil		
	Coconut		
	Peanut		
	Walnut		
Animal proteins	Cheese		Fish
	Egg		Meat
	Milk		Poultry

6. Iron absorption and its regulation

Dietary iron absorption requires its processing into a form in which it can be taken up by the intestinal mucosa. The most active site of absorption is the duodenum and the upper jejunum (50,51). It was also found that more iron is absorbed from the proximal small intestine than from the distal regions (52,53). The body's need for iron also influence absorption, and not all the iron presented in an absorbable form to the intestinal mucous membrane is necessarily transferred to the plasma. Absorption can be subdivided into two steps, namely the entry of iron into the mucosal cell from the

lumen of the gut – 'uptake' and its transfer from the mucosal cell into the body – 'transfer' (54). The exact mechanisms, which permit iron to enter the cell and regulate its transfer into the plasma, are unknown. Three major factors, which affect the absorption of iron from diet, are (1). the amount and availability of iron ingested (2) the behavior of the intestinal mucosal and (3) the body's need for iron. There are two pathways where by dietary iron enters the gastrointestinal mucosal cell according to the major forms of dietary iron, namely heme and non - heme iron. These two kinds of iron utilize two different receptors on the mucosal cells for their absorption. Heme iron is absorbed into the mucosal cell as the intact porphyrin complex via a luminal surface heme receptor (55). After mucosal uptake, it is rapidly catabolized by heme oxygenase (56) and the released iron then enters a common cellular pool. Its absorption is not affected by the many factors in the diet whereas non - heme iron is markedly influence by other ingredients (57). Solubilized non – heme iron present in the different constituents of a composite meal enters a common non - heme iron pool in the gastrointestinal lumen. Absorption occurs from this pool and different factors present in the meal such as the combination of enhancers and inhibitors influence the amount of iron available to the mucosal cell.

6.1 Heme iron absorption

The main source of heme iron is derived from meat, which forms only a small fraction of the dietary iron. Even in industrial countries heme iron constitutes approximately 10 – 15 percent of ingested iron, but because of its high bioavailability, may account for about one – third of the iron absorbed (58,59). When heme iron, ranging from 0.25 mg to 6 mg which is the physiological amounts, is eaten with food, percent absorption remains constant despite increasing heme iron content of the food

(60). The average absorption of heme iron in meat is usually around 25 percent but may vary from about 10 to 40 percent (61,62). When hemoglobin is ingested without other food constituents, a decreasing proportion of iron absorption from the hemoglobin occurs with increasing hemoglobin doses (63,64). Due to the linear relationship between the dose ingested and percent absorption of hemoglobin iron, increasing the dose results in an increase in the absolute amount of heme iron absorbed although the percentage absorbed declines. Pure heme molecules have lattice building qualities and bind with each other to form macromolecular polymers that are poorly absorbed (65). Hemoglobin iron is better absorbed than heme iron because of the enzymatic breakdown of globin into amino acids in the duodenum in which the primary amines coordinated with heme to maintain it as a small molecule in a monomeric state which is easily absorbed by mucosal cells. Absorption of heme iron is increases in patients with iron deficiency compared with normal subjects (63). Heme iron can be degraded and converted to non-heme iron if foods are cooked at a high temperature for too long (66,67).

6.2 Non – heme iron absorption

Non - heme iron is the major part of dietary iron. It is found in foods of both plant and animal origin. The physical form of ingested food and the retention time in the stomach may play a role in solubilizing non - heme iron from food. The hydrochloric acid content of gastric juice is also important for solubilization of non - heme iron (68). Absorption of ferric iron is more affected by achlorhydria than ferrous iron (69). Ferrous iron remains in solution at a higher pH than does ferric iron. Upon entry to the duodenum the pH rise results in rapid precipitation of ferric iron due to the formation of ferric oxyhydroxide. It is probable that iron can only be absorbed

into the cells and pass the mucosal membrane in its ferrous form (70). In duodenum not only the bicarbonate secretion and the resultant rise in pH reduced non-heme iron solubility but also pancreatic enzymes themselves may have an inhibitory effect on non-heme iron absorption (71). Once non - heme iron enters the common pool it is subject to the influence of the major dietary components namely protein, fat and carbohydrate together with a number of exogenous iron ligands such as ascorbic acid as an promoter and phytate as an inhibitor. Proteins and the exogenous ligands are the major influences that affecting bioavailability of non-heme iron. Fat and carbohydrate are relatively inert in terms of iron absorption (72).

6.3 Factor influencing non-heme iron absorption

The physicochemical form of iron affects iron absorption. It was found that ferrous iron is absorbed in greater quantities than ferric iron (73,74,75), dietary constituents which solubilize iron may enhance absorption, whereas compounds that cause precipitation or molecular aggregation of iron decrease absorption (76). Figure 1 shows the different types of dietary iron and a simplified model of the dietary non heme iron absorption. Native non heme iron is potentially available for absorption. Non- native forms are the extra iron of extrinsic origin such as fortification, and contamination and iron derived from food preparation equipment. These forms of iron are mainly unavailable for absorption. A number of dietary factors have been shown to influence the bioavailability of the potentially available non – heme iron.

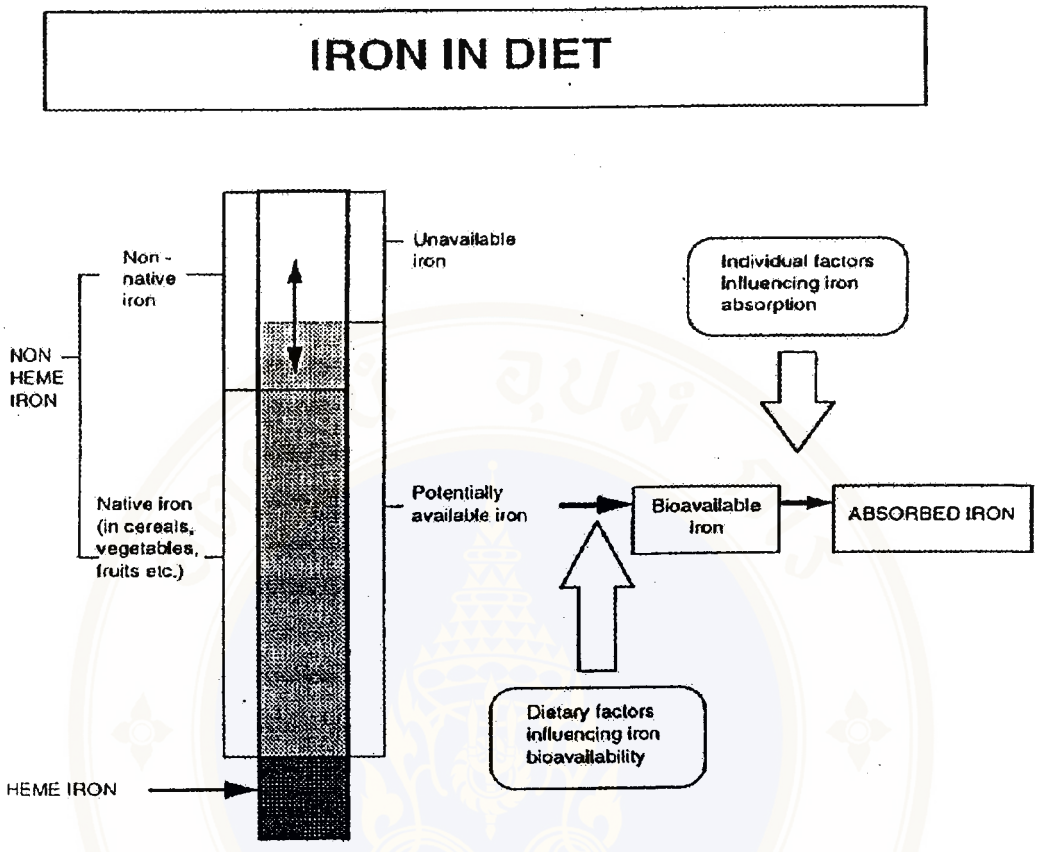

Figure 1. Iron in the diet and a simplified model of the dietary non- heme iron absorption.

6.3.1 Enchancers

The main enhancers of non - heme iron absorption are ascorbic acid, meat, poultry, fish, seafood and organic acids.

(a) Ascorbic acid

Ascorbic acid either in native or synthetic forms is a very powerful enhancer of non - heme iron. Native ascorbic acid in fruits, vegetables and juices increases iron absorption to the same extent as synthetic vitamin C. It potentiates non - heme iron absorption by two mechanisms, first by reduction of ferric

to ferrous iron in the stomach, second as a chelator in the stomach and upper small intestine. These both help to maintain iron in a soluble state by preventing its polymerization and binding to other inhibitory ligands. The enhancing effect of ascorbic acid is dose-effect relationship (77,78). The relationship was much affected by the composition of the meals (79). More ascorbic acid is needed to achieve a certain absolute increase in iron absorption if more inhibitors are present in a meal (80,81,82). Ascorbic acid in food may be partially or completely destroyed by cooking. The extent of destruction depends on the temperature, time and method of food preparation. Even prolong warming of food may destroy the ascorbic acid and therefore have a deleterious effect on the bioavailability of dietary iron (83).

(b) Meat, poultry, fish and seafood

Animal tissue protein has an enhancing properties on non-heme iron absorption. It has two roles in iron nutrition. First, it provides heme iron on average about 50 percent of iron in meat (beef, pork and lamb) with a variation form 10 to 70 percent. Second, it contains an unknown factor or meat factor that markedly enhances the absorption of both heme and non-heme iron. This enhancing factor is also present in poultry, fish and other seafood (5,84). The mechanism whereby meat factor facilitates non - heme iron absorption is not clear. It may be that meat factor has an iron solubilizing property to maintain iron in solution during progressive alkalization within the small intestine. Many attempts to isolate the potentiating meat factor of animal tissue protein have been made, since understanding the nature of this factor and its isolation could be of potential value in promoting iron absorption from less bioavailable foods.

Free amino acids, particularly the divalent amino acids asparagine, glycine and serine enhance iron absorption in the rat (85). In human, cysteine and cysteine – containing peptides have been identified as contributing to the meat factor (86,87).The cystine–containing peptides are stable in the gastrointestinal tract and their thiol groups tend to remain unoxidized. The myofibrillary proteins actin and myosin contain significant numbers of cysteine residues per molecule and it is possible that these proteins supply significant numbers of binding sites to iron in the gastrointestinal tract, thus maintaining it in solution (88).

(c) Organic acids

Besides ascorbic acid, other organic acids also have enhancing effects on non - heme iron absorption. Lactic acid has been identified as the factor that enhances iron absorption not only from sorghum and maize-derived beers in sub-Saharan Africa (89) but also from fermented cabbage (sauerkraut) (90). Citric acid present in citrus fruits and certain vegetables induces a significant three – to four-fold enhancing effect on non-heme iron absorption from a rice meal (91). Malic acid, a component of deciduous fruits, causes a significant two–fold enhancing affect on absorption from the rice meal and tartaric acid found in white wines displays a similar effect. (91).

6.3.2 Inhibitors

A number of factors have been identified which inhibit non-heme iron absorption from the common pool such as phytates, polyphenols and calcium.

(a) Phytate

Phytate is a salt of inositol hexaphosphates, acting as a storage of phosphate and minerals in grains, seeds, nuts, vegetables and fruits. It has a negative effect and in dose-dependent on iron absorption. Even rather small amounts, it has a marked effect (80). Bran has a very high content of phytate and a quantitative inhibiting effect occurs with increasing bran content of a meal (92). Unpolished rice has an inhibitory effect on non-heme iron absorption, which does not occur with polished rice and flour (81). The inhibiting effects present in beans, sorghum, oat products and in certain vegetables are partly related to their phytate content. (90,93). Removal of phytate from bran using endogenous phytase or dilute hydrochloric acid results in significantly improved absorption (94). The phytate content of rice may vary from region to region based on differences in soil nutrients and also on differences in milling practice (81). Wet milling of wheat reduces phytate content (82). The exact mechanism by the inhibition of phytate is unknown. The formation of diferric and tetraferric phytate complexes in the gastrointestinal tract renders the iron unavailable for absorption by the mucosal cell (95). The inhibitory effects of phytate are significantly counter-balanced by both meat and ascorbic acid (81,82).

(b) Polyphenols

The polyphenols or the polymers of phenolic compounds are chemical substances, which have one or more aromatic rings, bearing hydroxyl groups in different patterns. They are common constituents in a number of food items such as the beverages [tea (96), coffee (97), red wine (98)], vegetables [beans (99), peas (100), lentils (101), spinach and aubergine (90), spices (102), and pigmented

sorghum (103)]. Extremely high content is found in betel (104). They have significant inhibitory effects on non-heme iron absorption. Tea is a strong inhibitor (105,106). The inhibition of absorption by coffee and vegetables is related to their polyphenol content (6,107). The extent of inhibition is inversely related to the content of polyphenol and specifically to the nonhydrolysable condensed polyphenol (90). The mechanism of inhibition is thought to be due to the formation of complexes between the hydroxyl groups of the phenolic compounds and iron molecules. The inhibitory effects of the polyphenols can be overcome by the addition of ascorbic acid to foods. (6,82).

(c) Calcium

Inhibitory effect of calcium on iron absorption is dependent on the amount and form of calcium present in the meal (7,108,109). A condition for inhibition is that iron and calcium are in the same meal. When the intake of calcium and iron was separated in time (2 hours and 4 hours) no inhibition was seen (110). The exact inhibitory mechanism of calcium on absorption is not known. It was suggested that the inhibition be not localized to the gastrointestinal lumen but to the mucosal cell itself and to the common final transfer step for heme and non-heme iron (111). The magnitude of the calcium effect is dependent on the type of meal. Low bioavailability meals tend to have more effect if significant amounts of calcium are present (109).

7. Methods for measuring the bioavailability of iron from food

Iron absorption from the whole diet can be estimated by several methods. Each method has its limitations and advantages which are important considerations in the choice of method to study specific problems and in the interpretation of results. There are two main methods to study the bioavailability of dietary iron, namely in vivo and in vitro methods.

7.1 In vivo method

The most reliable method for determining dietary iron bioavailability by using radioiron labelled foods in human volunteer is an extrinsic tag method. The extrinsic tag method can be used to measure non – heme iron absorption from different meals, to search for and study various factors that may inhibitor or promote non-heme iron absorption, and to study the bioavailability of heme iron. The heme iron is biosynthetically labelled with radioiron-usually hemoglobin produced in iron deficient animals. The non - heme iron is labelled with inorganic radioiron tracer. The most significant advance in food iron absorption methodology was the demonstration that extrinsic radioiron added to food has essentially the same absorption as the food's intrinsic iron for both heme and non-heme iron (112). This method is based on the concept that food iron is absorbed from the two pools, the heme and the non-heme iron pool (9,113). Solubilized non-heme iron present in the different constituent of a composite meal enters a common non-heme iron pool in the gastrointestinal tract lumen. Absorption occurs from this pool and different factors present in the particular meal such as the combination of enhancers and inhibitors influence the amount of iron available to the mucosal cell. Similarly, heme iron

present in different food constituents enters a common pool form which heme iron is absorbed. The enhancing and inhibiting factors influencing absorption from the non-heme iron pool are, for the most part, without effect on the heme iron pool. From the use of ^{59}Fe and ^{55}Fe radioisotopes, absorption is estimated from the amount of radioiron incorporated into circulating red blood cells two to three weeks after ingesting the radioactive label. Incorporation of absorbed radioiron is approximately 80% in normal subjects. Comparing, iron absorption results from different studies and the significant variation in absorption between individuals, it is customary to measure absorption from a standard reference dose of inorganic radioiron containing 3 mg iron as ferrous ascorbate (48,114). The ratio of absorption from a given meal to absorption from the reference dose can then be used as a measure of the relative bioavailability of the non-heme iron in the meal. A more concrete measure of the latter is obtained by standardizing the test measurement to a reference value of 40%, the mean absorption value in subjects who are borderline iron deficient (113,115).

7.2 In vitro method

Several attempts have been made to design in vitro systems that will accurately predict absorption because of the radioisotopic evaluations of iron uptake are limited in human volunteers (12,16,116,). Most of the in vitro systems, ionizable, soluble or dialyzable iron was measured after an enzymatic digestion under physiological conditions (15,117). The extent of isotopic exchange between the added tracer (^{59}Fe) and dietary iron under simulating gastrointestinal digestion include acid hydrolysis, proteolytic digestion by pepsin and pancreatin is measured. Single food types or complex food mixtures can be evaluated and allowed examination of the effect of promotory and inhibitory ligands in modulation the amount of iron available

for absorption. Its results give a general prediction of food iron bioavailability rather than absolute levels of iron absorption (90,118). This may be due to effects of gastrointestinal motility, transit time, pH, enzyme concentrations and diffusion barriers, all affecting the proportion of ingested iron that is absorbed. However, in-vitro methods have an advantage in the elimination of variations in iron absorption which occur in vivo due to differences in iron status and day-to-day variability. The in vitro technique with some modifications from the previously published methods (11,12,16,116) was use for studying the dietary iron bioavailability in this thesis (119).

CHAPTER III

MATERIALS AND METHODS

Materials

Test meals: Thirty - four menus of common breakfast meals were studied. They were divided into 3 groups.

(1) Nine menus of boiled rice.

(2) Ten menus of simple steamed rice meals.

(3) Fifteen menus of more complex steamed rice meals (ie. with 2 food items)and the supplemental food.

In the groups of boiled and simple steamed rice, four kinds of drink were compared together with the breakfast. All the drinks are orange juice, milk, coffee and tea.

Six brands of the supplemental food, which are:

- Vitamax
- Vitamax – chocolate
- Monie Gold
- Monie Gold – chocolate
- Milo
- Ovaltine

were used to study in the fifteen menus of more complex steamed rice breakfast. Details of all menus were shown in Appendix E.

Radioactive iron : Iron - 59 (NEZ 037) as ferric chloride in 0.5 M Hydrochloric acid with specific activity of 10-20 mCi / mgFe obtained from DuPont NEN® Research Products, Boston, Massachusetts, USA, was used in this study.

Reagents

1. Reagents for determination of ionizable iron in breakfast meals

0.17 N HCl

Add 14.17 ml conc. HCl to a 1 litre volumetric flask and bring to volume with iron free water.

^{59}Fe radioactive

^{59}Fe as $FeCl_3$ was prepared 1μCi / 100 μl in 0.17 N HCl

1 % Pepsin in 0.17 N HCl

0.27 g Pepsin (Sigma G – 7000) was dissolved in 27 ml of 0.17 N HCl

0.1 M $NaHCO_3$

4.2 g $NaHCO_3$ (Sigma S – 8875) was prepared in 500 ml iron free water.

1% Pancreatin in 0.1 M $NaHCO_3$

0.42 g Pancreatin (Sigma P- 7545) was dissolved in 42 ml of 0.1 M $NaHCO_3$.

Bathophenanthroline solution

Dissolve 0.01079 g of 4, 7 – diphenyl –1,10 – phenanthroline in 52 ml ethyl alcohol and brought to130 ml with iron free water.

Acetate solution

Dissolve 55.1 g Sodium acetate granular ($NaC_2H_3O_3 .3H_2O$) in 100 ml iron free water.

4N Acetic acid

Dilute 23.5 ml concentrated acetic acid to 100 ml with iron free water.

Acetate buffer solution

4 ml of 4 N acetic acid was mixed with 96 ml acetate solution.

2. Reagents for determination of total iron in breakfast meals

Acetate buffer pH 4.75

450 g sodium acetate ($C_2H_3NaO_2.3HO_2$) was dissolved in deionized water, the pH adjusted to 4.75 using glacial acetic acid, the final volume was made up to one litre deionized water.

1 % Paranitrophenol

Dissolve 1g paranitrophenol in 100 ml iron free water.

Chromogen solution

200 mg disodium 4, 7 diphenyl 1, 10 – phenanthroline disulphonate was dissoved in deionized water, after adding 1 ml of thioglycolic acid solution (MW 92.12) the volume was adjusted to 100 ml.

Stock iron standard 1 mg/ml

100 mg of iron wire was dissolved in 2 ml concentrated hydrochloric acid, the volume was then adjusted to exactly 100 ml with deionized water..

Working iron standard 1 μg / ml

1 ml of stock iron standard was transferred into a volumetric flask and the volume was adjusted to one litre with deionized water.

3. Reagents for determination of phytate

Hydrochloric acid (HCl) 2.4 %

Add 54 ml conc HCl to a 1 litre volumetric flask and bring to volume with iron free water.

Sodium chloride solution (NaCl) 0.1 M and 0.7 M

NaCl 0.1 M : Dissolve 5.8443 g NaCl in 1 litre of iron free water.

NaCl 0.7 M : Dissolve 40.9101 g NaCl in 1 litre of iron free water.

Phosphate standard solution (80 μg P/ml)

Weigh 0.350 g of dried dessicated potassium acid phosphate (primary standard) into a 1 litre volumetric flask, add approximatety 500 ml of iron free water, add 10 ml of 10 N sulfuric acid (H_2SO_4) dilute to mark with iron free water.

Molybdate Solution

Dissolve 12.5 g ammonium hepta molybdate in 200 ml of iron free water. Transfer to 500 ml volumetric flask add 50 ml of 10 N sulfuric acid make to volume with iron free water.

Sulfonic acid reagent

Dissolve 0.16 g of 1– amino – 2 – napthal – 4 – sulfonic acid, and 1.92 g sodium sulfite (Na_2SO_3) and 9.60 g sodium bisulfite ($NaHSO_3$) in 90 ml iron free water. Transfer quantitatively to100 ml volumetric flask. Heat to dissolve if

necessary. Make to volume. (Freshly prepared weekly and store in brown bottle in refrigerator)

Na_2EDTA – NaOH reagent

Weigh 10.23 g disodium-ethylenediaminetetra - acetate (Na_2EDTA) and 75 g NaOH into 250 ml flask bring to volume with iron free water.

Standard phytic acid

Dissolve 26.6 mg of phytic acid in 25 ml iron free water.

4. Reagents for determination of iron-binding phenolic compounds

Acetate buffer 0.1 M, pH 4.4

Solution A: Add 11.55 ml acetic acid (CH_3COOH) to1000 ml iron free water.

Solution B: 16.40 g sodium acetate (CH_3COONa) was dissolved and diluted 1000 ml in iron free water.

Mix 305 ml of A with 195 ml of B, adjust the pH to 4.4 with sodium hydroxide (NaOH) and diluted to 1000 ml with iron free water.

50% Urea in acetate buffer

Dissolve 500 g urea (H_2NCONH_2) in 500 ml acetate buffer 0.1 M pH 4.4.

1% Arabic gum

Dissolve 1 g arabic gum in100 ml iron free water.

5 % FAS (Ferric Ammonium sulfate)

Dissolve 5 g FAS [$NH_4Fe(SO_4)_2.12H_2O$] in 100 ml 1M hydrochloric acid (HCl).

50% DMF. ACB

Mix 500 ml DMF (Dimethylformamide) with 500 ml 0.1 M, pH 4.4 ACB (Acetate buffer).

Food blank reagent

Prepare by mixing, just before use.

89 parts of 50 % urea solution in 0.1M acetate buffer.

10 parts of 1 % solution of arabic gum.

1 part of 1 M HCl.

FAS reagent (Iron – reagent)

Prepare by mixing, just before use.

89 parts of 50 % urea solution in 0.1 M acetate buffer.

10 parts of 1 % solution of arabic gum.

1part of 5 % solution of ferric - ammonium sulfate.

Standard stock - solution T.A

Dissolve 0.5 g of tannic acid (SIGMA Cat.No.T–0125) in 50%DMF- acetate and make up to 100 ml volume.

Standard stock - solution C

Dissolve 0.5 g of catechin (SIGMA Cat.No. C–1788) in 50 % DMF– acetate and make up to 100 ml volume.

Standard solutions

To prepare a calibration curve, pipet 1, 2, 4, 6 and 8 ml aliquots of tannic acid stock solution into 100 ml volumetric flasks and make up to volume with 50% DMF–acetate to produce standards at 25, 50, 100, 200 and 400 μg of tannic acid per ml. Pipet 1, 2, 4, 6, and 8 ml aliquots of catechin stock solution into100 ml volumetric

flasks and make up to volume with 50%DMF–acetate to produce catechin standards at 25, 50, 100, 200 and 400 μg per ml.Take 2 ml of each solution and add 8 ml FAS - reagent for colorimetric determination.

Note: Standard solutions and stock solutions must be stored in darkness then they are stable up to one week.

This analytical method was not determine the total phenolic compounds in foods, but measures, in vitro, only those phenolic compounds that might inhibit iron absorption in vivo. The phenolic compounds which have iron – binding structures (galloyl and catechol groups) had proved to have such effect.This method thus measures the content of extractable phenolics with galloyl and catechol groups.

Instruments and special glasswares

Instruments and special glasswares were listed as follows:

- Food Homogenizer, Moulinex France,
- Shaker Bath, Cat No 6250, Eberbach Corporation, Ann Arbor, Michigan.
- Shaker, New Brunswick Scientific, Model R –2, Serial No.382260, Edison, N – J., USA.
- Vortex –mixer, Vortex – Genic 2, Scintific Industries, Inc., Bohemia, N.Y. 11716, USA.
- IEC Centra – 8 Centrifuge, Model 2477, International Equipment Company, 300 second Avenue, Needharm Heights, MA 02194, USA.

- Spectrophotometer Spectronic 21, Milton Ray Company, Analytical Products Division (Formerly a division of BAUSCH & LOMB) 820 Lindess Avenue, Rochester, N.Y.14625.
- Digital pH meter, Model SP–7, Suntex Instruments Co., Ltd., TAIWAN.
- Magnetic stirer, Ikamag REC–G Janke & Kunkel Gmb H.U.CoKG, IKA–Werk 7813 staufen.
- CompuGamma Gamma Counter, Model 1282, LKB Wallac, Wallac Oy, P.O. Box 10, 20101 Turku 10, Finland.
- Mettler Electric Balance, Mettler P1210, Mettler, Zurich, Switzerland.
- Sauter Electric Balance, August Sauter GmbH D–7470 Albstadt 1–Ebingen.
- HAVARD TRIP Balance, OHAUS, USA.
- Econo–column, Chromatography Columns, Catalog No. 737–1232 Bio–Rad Laboratories, 32nd & Griffin Avenue, Richmond, California 94804.
- Labconco Micro–Kjeldahl Digestors, Model 60301 – 01, Labconco Corporation, 8811 Prospect, Kansas City. Mo. 64132.
- The stainless steel Perchloric Acid Hood. Labconco Model 47807, Labconco Corporation, 8811 Prospect, Kansas City, Mo. 64132.
- Eppendorf Pipette 300, Eppendorf Geratebau, Netheler + Hinz GmbH, Postfach 650670, 2000 Hamburg 65.
- Anion Exchange resin AG – 1 X4. 100 – 200 mesh chloride form, Biorad Lab. Cat No. 140 – 1341.
- Micro Kjeldahl Flask (100 ml), Labconco Model 60375.
- Whatman filter paper No. 541, cat. No.1541125.

Methods

Chemical composition of meals

The breakfast meal sample was homogenized in a food blender with deionized water to a creamy consistency. After blending, the homoginized sample was weighed for analyzing of ionizable iron, total iron, total phosphorus, phytate and iron – binding phenolic compound.

Determination of ionizable iron

An in vitro method for the determination of availability of nonheme iron from food described by Sritongkul (16,119) was used in this study.

Detailed in Appendix A.

Determination of total iron

The total iron content in meal was determined by the recommended wet digestion method (120).

Detailed in Appendix B.

Determination of total phosphorus and phytate

Phosphorus and phytate were analysed using anion exchange procedure as described by Harland and Oberleas (121).

Detailed in Appendix C.

Determination of iron – binding phenolic compound (Tannin)

Determination of iron binding phenolic compound was performed, using modified FAS method (103).

Detailed in Appendix D.

Statistical analysis

All calculations for descriptive statistics, graphic presentations and statistical analyses were made by using SPSS for windows. Differences in meal compositions and the percentage of iron ionizable, percentage of estimated iron absorption, expect absorbed iron within and between groups were evaluated by nonparametric test (Kruskal – Wallis Test). Differences among the means of those variables were determined by nonparamatric test (Mann – Whitney Test) at $p < 0.05$.

CHAPTER IV

RESULTS

1. General characteristics of test meals

1.1 Total iron content, iron density and energy intake

1.1.1 Boiled rice as basal breakfast

Table 5 shows mean total iron content in 9 meals assigned as basal breakfast (boiled rice), basal breakfast with orange juice, milk, coffee and tea ranged from 0.99 to 1.14 mg per meal, the mean ± SEM were 0.99 ± 0.15, 1.14 ± 0.15, 1.07 ± 0.14, 1.12 ± 0.15 and 1.03 ± 0.15 mg per meal respectively. The medians were 0.91, 1.06, 0.94, 1.0 and 0.91 mgFe/meal respectively. The mean and median of the iron density were 2.91 and 2.03 mgFe/1000 kcal. These values were much lower than the recommended value of 6 to 12 mgFe.1000 kcal. The mean caloric intake was 453.1 kcal per meal which is quite usual for breakfast meal.

1.1.2 Steamed rice as basal breakfast

The mean total iron content in 10 meals assigned as basal breakfast (steamed rice), basal breakfast with orange juice, milk, coffee and ranged from 3.43 to 3.61 mg per meal, the mean + SEM were 3.46 ± 1.65, 3.61 ± 1.65, 3.55 ± 1.65, 3.60 ± 1.65 and 3.43 ± 1.61 mg per meal respectively as shown in Table 6. The median were 1.1, 1.26, 1.2, 1.28 and 1.15 mgFe/meal respectively. The median

of the iron density was 2.88 mgFe/1000 kcal. The mean ± SEM of 7.73 ± 3.47 mg/1000 kcal although lay within the normal range, the median was down below the accepted standard, 6 – 12 mg/1000 kcal. The mean caloric intake was 431.1 mg/meal.

1.1.3 Steamed rice with supplement food

The mean ± SEM of the total iron content per meal and iron density per 1000 kcal were 3.22 ± 0.48 and 7.01 ± 1.26 mg respectively as shown in Table 7 and Figure 6. The medians of the iron intake and iron density were 2.66 mg/meal and 5.52 mg/1000 kcal. The mean iron density of 7.01 mg/1000 kcal was within the recommended range. Figure 2 shows the means and medians of iron density among the three test meals. The total energy intake per meal was 484.07 kcal.

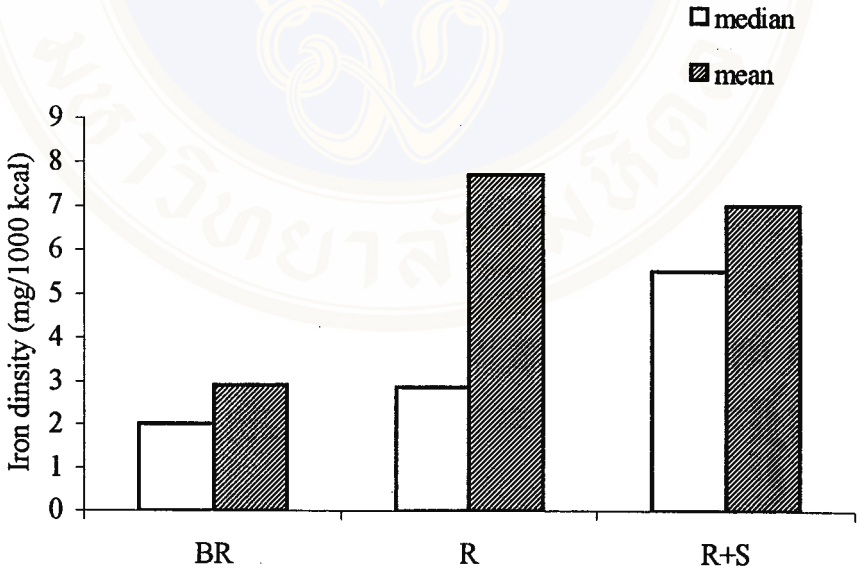

Figure 2. Means and medians of iron density (mg per 1000 kcal) of 3 types of basal breakfast.

BR = Boiled rice, R = Steamed rice, R+S = Steamed rice + Supplement food

TABLE 5. Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron and iron density in boiled rice meals with different drinks.

No.	ID	Wt (g)	kcal	mg / meal		Total Fe (mg / meal)					Fe density
				Phos	Phy	B	O	M	C	T	mg/1000kcal
1	1	500	321.5	49.50	3.41	0.88	1.03	0.94	0.94	0.91	2.74
2	2	500	365.8	111.1	8.85	0.53	0.68	0.63	0.63	0.58	1.45
3	3	500	437.2	165.1	11.6	1.66	1.81	1.70	1.69	1.83	3.80
4	4	500	552.0	160.3	2.99	1.12	1.27	1.12	1.25	1.12	2.03
5	5	500	535.6	234.1	4.50	0.91	1.06	1.00	1.00	1.00	1.70
6	6	500	369.4	194.6	10.7	0.91	1.06	0.91	1.01	0.85	2.46
7	7	500	327.4	165.1	14.8	0.48	0.63	0.59	0.70	0.48	1.47
8	8	500	535.6	204.1	2.97	0.70	0.85	0.91	0.82	0.82	1.31
9	9	500	633.4	249.4	1.50	1.76	1.91	1.79	2.02	1.72	2.78
Mean		500	453.1	170.4	6.81	0.99	1.14	1.07	1.12	1.03	2.19
Median		500	437.2	165.1	4.50	0.91	1.06	0.94	1.00	0.91	2.03
SD		0	113.9	61.55	4.74	0.45	0.45	0.42	0.46	0.46	0.82
SEM		0	37.99	20.52	1.58	0.15	0.15	0.14	0.15	0.15	0.27

B = Basal meal (Boiled rice)

O = Basal meal + orange juice

M = Basal meal + milk

C = Basal meal + coffee

T = Basal meal + tea

TABLE 6. Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron and iron density in simple steamed rice meals with different drinks.

No	ID	Wt (g)	kcal	mg / meal		Total Fe (mg / meal)					Fe density
				Phos	Phy	B	O	M	C	T	mg/1000kcal
1	10	500	492.5	224.0	6.03	1.12	1.27	1.21	1.32	1.16	2.27
2	11	500	407.8	205.2	6.03	0.88	1.03	1.00	1.02	1.00	2.16
3	12	600	377.2	502.8	10.9	1.09	1.24	1.19	1.24	1.13	2.89
4	13	600	518.6	351.9	9.11	15.8	15.9	15.9	15.9	15.9	30.45
5	14	500	328.8	234.1	12.3	0.94	1.09	0.94	1.03	1.00	2.86
6	15	500	388.6	433.0	18.9	10.3	10.4	10.4	10.4	9.46	26.45
7	16	550	543.8	316.4	6.59	0.77	0.92	0.87	0.95	0.81	1.42
8	17	550	390.0	152.5	6.59	0.90	1.05	1.03	1.02	1.01	2.31
9	18	550	381.2	316.6	16.7	1.23	1.38	1.34	1.41	1.27	23.23
10	19	600	482.1	243.0	10.8	1.58	1.73	1.68	1.66	1.62	3.28
Mean		545	431.1	297.9	10.4	3.46	3.61	3.55	3.60	3.43	7.73
Median		550	398.9	279.7	9.96	1.11	1.26	1.2	1.28	1.15	2.88
SD		43.8	71.98	1.8.6	4.54	5.22	5.22	5.22	5.22	5.10	10.97
SEM		13.8	22.76	34.34	1.44	1.65	1.65	1.65	1.65	1.61	3.47

B = Basal meal (Steamed rice)

O = Basal meal + orange juice

M = Basal meal + milk

C = Basal meal + coffee

T = Basal meal + tea

TABLE 7. Showing general characteristics of test meals: weight, energy intake, total phosphorus, phytate, total iron, iron density and expected absorbed iron in the more complex steamed rice meals with different supplemented foods.

No	ID	Wt (g)	kcal	mg / meal				Fe density mg/1000kcal
				Phos	Phy	Total Fe	Absorbed iron*	
1	20	600	605.1	502.8	63.30	3.30	0.36	5.45
2	21	600	547.8	531.8	51.04	7.07	1.42	12.91
3	22	600	507.8	290.8	27.80	1.41	0.10	2.78
4	23	600	492.0	280.9	21.70	1.24	0.25	2.52
5	24	600	576.8	336.7	19.31	2.66	0.28	4.61
6	25	600	435.5	290.8	11.95	2.05	0.38	4.71
7	26	600	374.2	280.9	12.87	2.23	0.16	5.96
8	27	600	501.2	353.7	24.72	4.33	0.60	8.64
9	28	600	535.1	608.6	18.71	3.74	0.36	6.99
10	29	600	383.8	417.5	24.72	2.12	0.40	5.52
11	30	600	347.9	224.4	30.93	7.58	0.80	21.78
12	31	600	428.4	411.5	33.06	3.28	0.44	7.66
13	32	600	619.5	321.4	16.75	2.16	0.14	3.49
14	33	600	550.6	576.7	26.77	2.20	0.28	3.99
15	34	600	355.3	488.3	22.68	2.88	0.33	8.11
Mean		600	484.1	394.5	27.09	3.22	0.42	7.01
Median		600	501.2	336.7	24.7	2.66	0.36	5.52
SD		0	91.15	121.2	13.80	1.86	0.33	4.88
SEM		0	23.53	31.30	3.56	0.48	0.08	1.26

* Expected absorbed iron calculated from $y = 0.3145 + 0.4312X$ (12).

1.2 Total phosphorus and phytate contents

The mean ± SEM of the phosphorus contents for the 2 basal breakfasts (boiled rice and steamed rice) were 170 ± 20.52 and 297.96 ± 34.34 mg per meal and the mean ± SEM of the phytate contents were 6.81 ± 1.58 and 10.41 ± 1.44 mg per meal respectively (Table 5 and 6). For basal meal with supplement food, the mean ± SEM of the total phosphorus and phytate contents were 394.46 ± 31.30 and 27.09 ± 3.56 mg per meal as shown in Table 7. Summary of general characteristics of the 3 groups of test meals was shown in Table 8.

TABLE 8. Summary of the general characteristics of test meals.

Sample	N	kcal	mg / meal			Iron density mg/meal
			Phos	Phy	Fe	
Boiled rice	9	453.1	170.36	6.81	0.99 (0.91)*	2.19, (2.03)*
Steamed rice	10	431.1	297.96	10.41	3.46 (1.1)*	7.73, (2.88)*
Rice + supplement food	15	484.1	394.46	27.09	3.22 (2.66)*	7.01, (5.52)*

* medians in parenthesis

As shown in Table 8, the boiled rice breakfast showed the lowest mean iron intake, 0.99 mg/meal and also the lowest mean iron density, 2.19 mg/1000 kcal. The second breakfast although had higher mean iron intake and iron density, 3.46 mg/meal and 7.73 mg/1000 kcal, the medians were in the same magnitude as the first group 0.91 VS 1.11 mg/meal and 2.03 VS 2.88 mg/1000 kcal. The third breakfast meals with supplement foods had higher mean and median of the observations than the first-two groups, 7.01 mg/meal and 5.52 mg/1000 kcal. However, their caloric intakes were within the same magnitude, 453.1, 431.1 and 484.1 kcal respectively.

Statistical analysis by nonparametric Kruskal Wallis test using SPSS for windows showed that the iron content, iron density, phosphorus and phytate contents among the three rest meals were significant different at $p < 0.001$ except for the caloric intake. Test statistics were summarized in Table 9.

TABLE 9. Summary of the test statistic by Kruskal – Wallis test of the 3 groups of breakfast.

Statistics	Fe	Fe density	Phosphorus	Phytate	kcal
Chi-square	15.449	14.568	17.215	22.603	1.630
P -value	< 0.001	< 0.001	< 0.001	< 0.001	0.443

Comparisons between 2 groups by nonparametric Mann-Whitney test were summarized in Table 10.

TABLE 10. Summary of the test statistic (Mann-Whitney Test) for total iron, iron density, total phosphorus and total phytate contents between 2 meals.

Meal	Fe	Fe density	Phosphorus	Phytate
1 – 2	NS	NS	< 0.01	NS
1 – 3	< 0.001	< 0.001	< 0.001	< 0.001
2 – 3	< 0.05	< 0.05	NS	< 0.001

NS = Not significant, 1 = Basal breakfast (boiled rice), 2 = Basal breakfast (steamed rice), 3 = Basal breakfast (steamed rice with supplement food)

1.3 Determination of tannin and catechin in drinks and supplement food

Table 11 shows the contents of tannin and catechin per meal in coffee, tea, milo, ovaltine and chocolate in supplement food. The highest tannin content was found in tea. Distribution of the contents was shown in Figure 3.

Table 11. Contents of tannin and catechin in coffee, tea and chocolate in supplement food.

Drink	mg/g		mg/meal	
	Tannin	Catechin	Tannin	Catechin
Coffee	12.55	97.40	94.13	730.50
Tea	47.35	133.20	415.80	383.40
Milo	5.30	13.70	79.50	205.50
Ovaltine	4.30	11.0	64.50	165.00
Chocolate	30.05	40:60	150.00	203.00

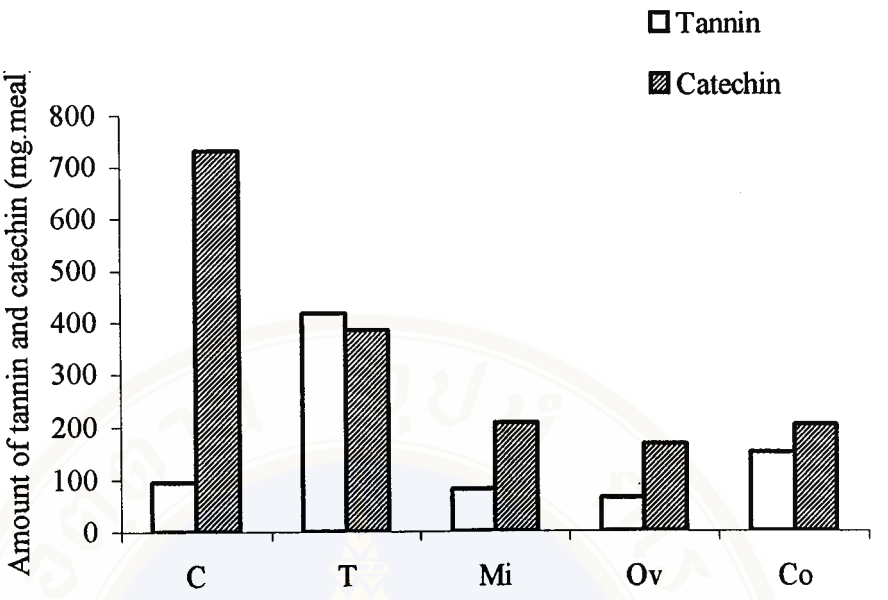

Figure 3. Distribution of the contents of tannin and catechin (mg/meal) in coffee (c), tea (T), milo (Mi), ovaltine (Ov) and chocolate (Co).

2. Determination of food iron ionizability and percentage of the estimated iron availability

The percentage of the ionizable iron (%II) and the estimated iron absorption (% EIA) from 3 different group of test meals were shown in Table 12, 13, 14(a) and 14(b). The calculation of the percentage of estimated iron absorption was based on the correlation between percent ionizable iron and percent in-vivo absorption, food iron absorption was estimated from the predication equation $y = 0.3145 + 0.4312\ x$, where y is the estimated percent iron absorption and x is the percent ionizable iron at pH 5.8.

2.1 Boiled rice as basal breakfast

The effect of adding a variety of drinks into boiled rice breakfast was shown in Table 12. The mean $\pm$ SEM of the percent ionizable iron in boiled rice meal

and boiled rice plus orange juice, milk, coffee and tea were 11.7 ± 2.1, 25.3 ± 3.51, 21.3 ± 3.46, 7.08 ± 0.62 and 1.57 ± 0.08 respectively. The percentage of the estimated iron absorption (mean ± SEM) being 8.43 ± 1.70, 20.85 ± 3.47, 8.60 ± 1.41, 6.47 ± 0.99 and 1.28 ± 0.17 percent respectively. As shown in Table 12, orange juice improved food iron availability approximately by 2.5 time (+148%), milk showed almost no effect (+2%), coffee and tea decreased iron absorption by 23% and 85% respectively.

2.2 Steamed rice as basal breakfast

As shown in Table 13, the mean ± SEM of the percent ionizable iron for basal meal alone and basal meal with orange juice, milk, coffee and tea were 14.85 ± 3.08, 33.16 ± 5.07, 22.39 ± 3.61, 6.41 ± 0.26, 1.42 ± 0.11 respectively and the mean ± SEM of the percentage of estimated iron absorption being 11.17 ± 2.63, 22.01 ± 3.41, 10.64 ± 1.52, 4.48 ± 0.27 and 1.37 ± 0.13 percent respectively. Orange juice improved iron absorption almost 2 times (97%), milk, coffee and tea decreased iron absorption by 5%, 60% and 88% respectively.

2.3 Steamed rice meals with supplement foods

As shown in Table 14(a), the mean ± SEM of the percent ionizable iron of basal meal alone and basal meal with supplement foods, Vi, Vi-c, Mo, Mo-c, Mi and Ov were 14.83 ± 1.7, 18.81 ± 1.64, 9.31 ± 1.54, 15.0 ± 2.05, 5.41 ± 1.23, 6.78 ± 1.25 and 5.63 ± 1.17% respectively. It was observed that chocolate drinks,Vi-c, Mo-c, Mi and Ov decreased the percent of food iron ionizability remarkedly. The percentage reduction was found to be 37%, 64%, 54% and 62% respectively.

The percentage of estimated iron absorption was in Table 14(b). The mean ± SEM were 12.86 ± 1.22, 18.0 ± 1.07, 12.8 ± 0.69, 12.6 ± 1.12, 7.3 ± 0.56, 9.9

± 0.56 and 7.9 ± 0.48% for basal meal alone, basal meal with Vitamax, Vitamax-chocolate, Monie Gold, Monie Gold-chocolate, milo and ovaltine respectively.

Statistical analysis using nonparametric Mann-Whitney test showed that there was no significant different in either the percent ionizable or the percentage of the estimated absorbed iron among these 3 groups of breakfast at the significant level of 0.05.

TABLE 12. The percentage of ionizable iron and estimated iron absorption in boiled rice meals with different drinks.

No	ID	% II					% EIA				
		B	O	M	C	T	B	O	M	C	T
1	1	16.4	34.6	13.0	6.20	2.00	13.1	24.6	5.01	8.10	1.70
2	2	16.9	29.0	29.3	5.70	1.20	12.7	27.5	10.3	5.19	0.97
3	3	9.20	11.4	11.1	8.10	1.60	2.94	5.66	4.71	7.17	1.74
4	4	8.00	17.9	13.3	10.5	1.90	3.60	11.2	6.10	12.4	2.17
5	5	6.90	30.1	38.4	9.50	1.60	9.90	31.6	16.1	9.10	0.92
6	6	24.5	31.8	32.6	5.90	1.30	16.7	26.2	11.4	4.30	0.80
7	7	10.6	32.5	24.9	5.10	1.50	8.70	28.4	12.4	3.60	0.70
8	8	6.40	34.1	18.9	6.60	1.50	6.20	27.2	7.90	4.20	1.10
9	9	6.10	6.70	10.3	6.10	1.50	2.10	5.30	3.50	4.20	1.40
Mean		11.7	25.3	21.3	7.08	1.57	8.43	20.9	8.60	6.47	1.28
SD		6.29	10.5	10.4	1.86	0.25	5.11	10.4	4.22	2.96	0.50
SEM		2.10	3.51	3.46	0.62	0.08	1.70	3.47	1.41	0.99	0.17
					% change		0	+148	+2	-23	-85

TABLE 13. The percentage of ionizable iron and estimated iron absorption in sample steamed rice meals with different drinks.

No	ID	% II					% EIA				
		B	O	M	C	T	B	O	M	C	T
1	10	9.10	20.4	9.20	5.30	2.10	6.80	16.80	7.89	2.60	1.90
2	11	6.40	11.7	9.70	5.40	1.10	2.85	5.56	3.88	4.10	2.03
3	12	36.0	45.8	44.0	6.50	1.40	27.43	32.80	20.01	4.57	1.44
4	13	13.2	31.2	18.4	7.10	1.30	16.73	26.86	9.92	5.41	1.11
5	14	6.70	31.3	18.3	6.10	1.20	6.88	29.69	11.54	4.10	0.89
6	15	7.70	30.2	18.0	6.50	1.00	3.64	27.70	9.54	4.53	0.84
7	16	15.9	30.1	27.8	7.10	1.90	8.33	13.37	10.68	5.09	1.68
8	17	22.6	67.2	37.1	7.90	1.50	17.40	33.77	15.04	5.29	1.14
9	18	23.2	44.9	26.6	6.40	1.50	18.47	27.95	13.38	5.22	1.39
10	19	7.70	18.8	14.8	5.80	1.20	3.13	5.58	4.49	3.91	1.26
Mean		14.85	33.16	22.39	6.41	1.42	11.17	22.01	10.64	4.48	1.37
SD		9.74	16.03	11.43	0.81	0.35	8.32	10.79	4.81	0.86	0.40
SEM		3.08	5.07	3.61	0.26	0.11	2.63	3.41	1.52	0.27	0.13
					% change		0	+97	-5	-60	-88

TABLE 14(a). The percentage of ionizable iron in the more complex steamed rice meals with different supplemented foods.

No	ID	% II						
		B	Vi	Vi – c	Mo	Mo - c	Mi	Ov
1	20	6.70	12.8	5.70	6.50	2.70	1.80	2.30
2	21	27.5	27.2	18.5	29.5	13.3	14.1	13.3
3	22	12.8	19.9	8.30	14.3	1.20	3.90	1.60
4	23	10.3	12.0	3.10	8.20	1.70	3.00	2.30
5	24	8.00	17.6	4.80	7.50	1.80	3.40	1.90
6	25	7.70	14.3	2.20	5.30	0.70	8.30	4.80
7	26	17.9	20.2	8.90	19.3	2.80	6.50	4.10
8	27	22.7	23.7	18.1	20.6	11.2	13.4	12.0
9	28	15.0	20.7	11.0	9.20	3.10	2.10	2.50
10	29	12.8	19.3	2.80	13.8	1.70	2.90	1.60
11	30	9.90	11.0	8.30	7.60	5.80	8.00	9.90
12	31	23.4	23.6	21.3	24.0	16.0	18.0	14.7
13	32	15.1	33.5	8.50	17.0	8.00	4.30	3.90
14	33	10.1	12.8	5.60	13.9	4.30	5.50	4.00
15	34	22.6	13.6	12.5	28.3	6.90	6.50	5.60
Mean		14.83	18.81	9.31	15.0	5.41	6.78	5.63
SD		6.57	6.37	5.98	7.94	4.77	4.86	4.53
SEM		1.70	1.64	1.54	2.05	1.23	1.25	1.17
% change		0	+27	-37	+1	-64	-54	-62

B = Basal meal, Vi = Basal meal + Vitamax, Vi – c = Basal + Vitamax – chocolate, Mo = Basal meal + Monie Gold, Mo – c = Basal meal + Monie Gold – chocolate, Mi = Basal meal + Milo, Ov = Basal meal + Ovaltine

TABLE 14(b). The estimated iron absorption in the more complex steamed rice meals with different supplement foods.

No	ID	% EIA						
		B	Vi	Vi – c	Mo	Mo - c	Mi	Ov
1	20	10.84	15.5	9.6	7.1	8.44	7.73	7.96
2	21	20.11	21.2	15.6	19.6	9.71	14.2	11.6
3	22	7.43	23.3	14.2	8.98	3.25	9.67	5.37
4	23	20.45	20.0	15.4	19.1	7.6	13.4	8.89
5	24	10.71	16.0	10.0	11.2	4.31	10.6	5.48
6	25	18.77	21.8	14.1	15.3	6.69	11.5	8.18
7	26	7.30	14.0	9.5	7.92	5.56	7.03	6.19
8	27	13.94	14.8	10.6	13.3	7.42	9.65	7.7
9	28	9.71	15.2	11.7	8.57	6.49	7.66	7.18
10	29	19.03	25.3	18.6	19.3	9.48	9.45	8.16
11	30	10.62	11.9	13.6	9.54	8.65	9.99	11.5
12	31	13.34	14.3	10.7	13.5	9.11	11.4	8.7
13	32	6.52	15.6	10.5	7.77	6.23	7.21	6.26
14	33	12.65	23.3	13.9	13.6	5.46	11.1	6.95
15	34	11.44	17.2	13.7	13.5	11.1	8.59	8.87
Mean		12.86	18.0	12.8	12.6	7.3	9.9	7.9
SD		4.71	4.14	2.66	4.33	2.16	2.15	1.86
SEM		1.22	1.07	0.69	1.12	0.56	0.56	0.48

3. Estimation of iron absorbed from 3 groups of breakfast meals

Tables 15, 16, 17 and Figure 4, 5 and 6 show the expected amount of absorbed iron from the 3 different breakfast meals. Since there was no significant difference in the percent ionizable iron and the percent of estimated absorbed iron among the 3 breakfasts, the amount of absorbed iron will depend only on the total iron contents in the test meals. The iron contents of meals in the 3 groups of breakfasts were not normally distributed, some meals had extremely high iron content as they contained haemoglobin iron in the food composition such as cooked blood/(pork). Kruskal Wallis test showed that there was a significant difference in the amount of iron expected to be absorbed among the 3 groups at $p < 0.001$. Statistical analysis using Mann-Whitney test showed that there was no significant different in the amount of iron absorbed between the first 2 breakfasts, boiled rice with drinks and steamed rice with drinks at a significant level of 0.05 but the estimated absorbed iron in groups 3, breakfast with supplement food was significantly higher than the first 2 groups at $p< 0.05$.

The effects of soft drinks and supplement food on food iron availability form breakfast meals were shown by the discrepancy in p-values calculated using Mann-Whitney test. Table 18(a) gives p-value from the comparisons between boiled rice breakfast with and without drinks to determine whether there are any differences among their means, Table 18(b) is a comparison among the steamed rice breakfast with and without soft drinks and table 18(c) is a comparison among the steamed rice breakfast with and without supplement foods.

TABLE 15. Estimation of the amount of iron expected to be absorbed from boiled rice meals with different drinks.

No	ID	Expected absorbed iron (mg / meal)				
		B	O	M	C	T
1	1	0.12	0.25	0.05	0.08	0.02
2	2	0.07	0.19	0.07	0.03	0.006
3	3	0.05	0.10	0.08	0.12	0.03
4	4	0.04	0.14	0.07	0.16	0.02
5	5	0.09	0.33	0.16	0.09	0.009
6	6	0.15	0.28	0.10	0.04	0.007
7	7	0.04	0.18	0.07	0.03	0.003
8	8	0.04	0.23	0.07	0.03	0.009
9	9	0.04	0.10	0.06	0.08	0.02
Mean		0.07	0.20	0.08	0.07	0.01
SD		0.04	0.08	0.03	0.04	0.009
SEM		0.01	0.03	0.01	0.01	0

TABLE 16. Estimation of the amount of iron expected to be absorbed from simple steamed rice meals with different drinks.

No	ID	Expected absorbed iron (mg / meal)				
		B	O	M	C	T
1	10	0.08	0.21	0.09	0.03	0.02
2	11	0.03	0.06	0.04	0.04	0.02
3	12	0.30	0.41	0.24	0.06	0.02
4*	13	2.64	4.28	1.58	0.86	0.18
5	14	0.06	0.32	0.11	0.04	0.008
6	15	0.37	2.89	0.99	0.47	0.08
7	16	0.06	0.12	0.09	0.05	0.01
8	17	0.16	0.35	0.15	0.06	0.01
9	18	0.23	0.39	0.18	0.07	0.02
10	19	0.05	0.10	0.08	0.07	0.02
Mean		0.15	0.54	0.22	0.10	0.02
SD		0.12	0.89	0.30	0.14	0.02
SEM		0.04	0.30	0.10	0.03	0.006

* Extremely high result not included in the calculation

TABLE 17. Estimation of the amount of iron expected to be absorbed from the complex steamed rice meals with supplement foods.

No	ID	Expected absorbed iron (mg/meal)						
		B	Vi	Vi-c	Mo	Mo-c	Milo	Ov
1	20	0.36	0.51	0.32	0.23	0.28	0.26	0.26
2	21	1.42	1.50	1.10	1.39	0.69	1.00	0.82
3	22	0.10	0.33	0.20	0.13	0.05	0.14	0.08
4	23	0.25	0.25	0.19	0.24	0.09	0.17	0.11
5	24	0.28	0.43	0.27	0.30	0.11	0.28	0.15
6	25	0.38	0.45	0.29	0.31	0.14	0.24	0.17
7	26	0.16	0.31	0.21	0.18	0.12	0.16	0.14
8	27	0.60	0.64	0.46	0.58	0.32	0.42	0.33
9	28	0.36	0.57	0.44	0.32	0.24	0.29	0.27
10	29	0.40	0.54	0.39	0.41	0.20	0.20	0.17
11	30	0.80	0.9	1.03	0.72	0.66	0.76	0.87
12	31	0.44	0.47	0.35	0.44	0.30	0.37	0.29
13	32	0.14	0.34	0.23	0.17	0.13	0.16	0.14
14	33	0.28	0.51	0.31	0.30	0.12	0.24	0.15
15	34	0.33	0.50	0.39	0.39	0.32	0.25	0.26
Mean		0.42	0.55	0.41	0.41	0.25	0.33	0.28
SD		0.33	0.31	0.28	0.31	0.19	0.24	0.24
SEM		0.09	0.08	0.07	0.08	0.05	0.06	0.06

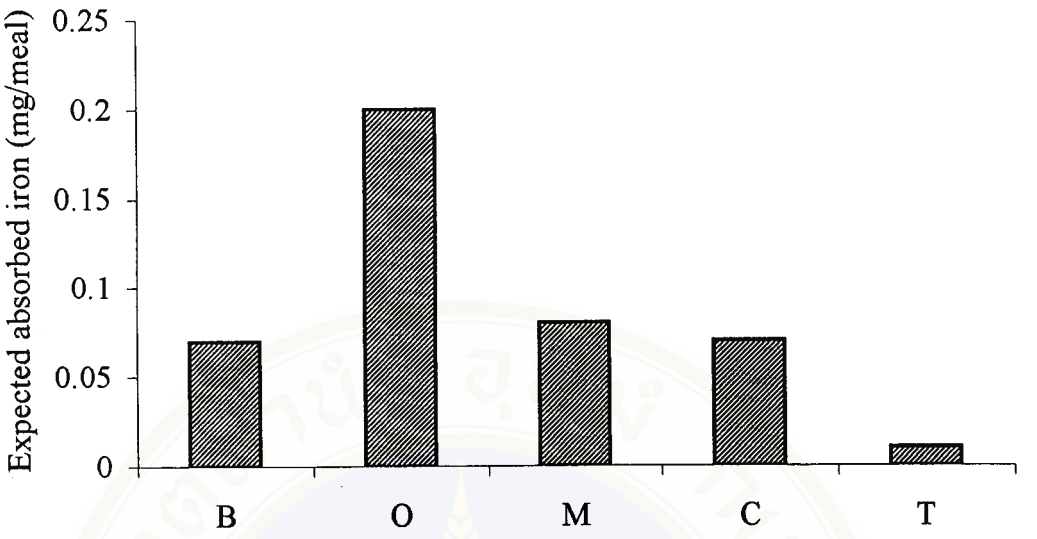

Figure 4. Estimation of the amount of iron expected to be absorbed from boiled rice meals with different drinks.

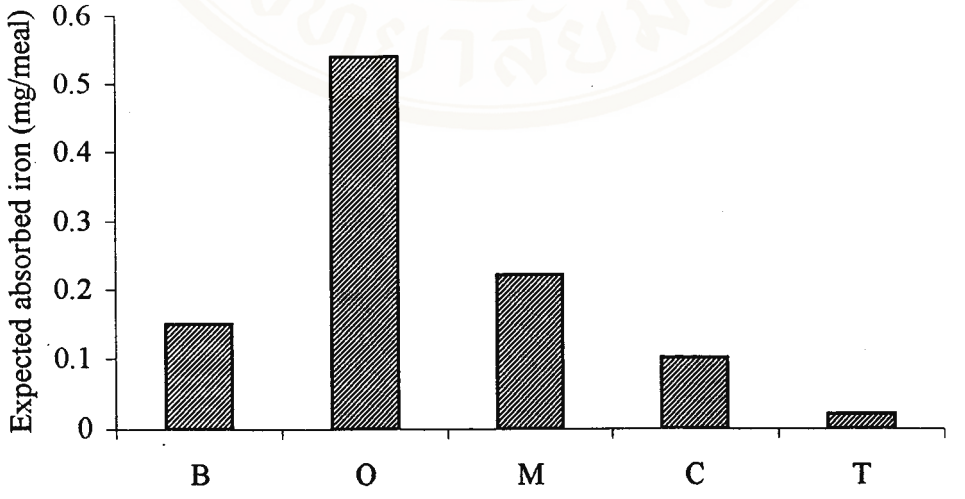

Figure 5. Estimation of the amount of iron expected to be absorbed from simple steamed rice meals with different drinks.

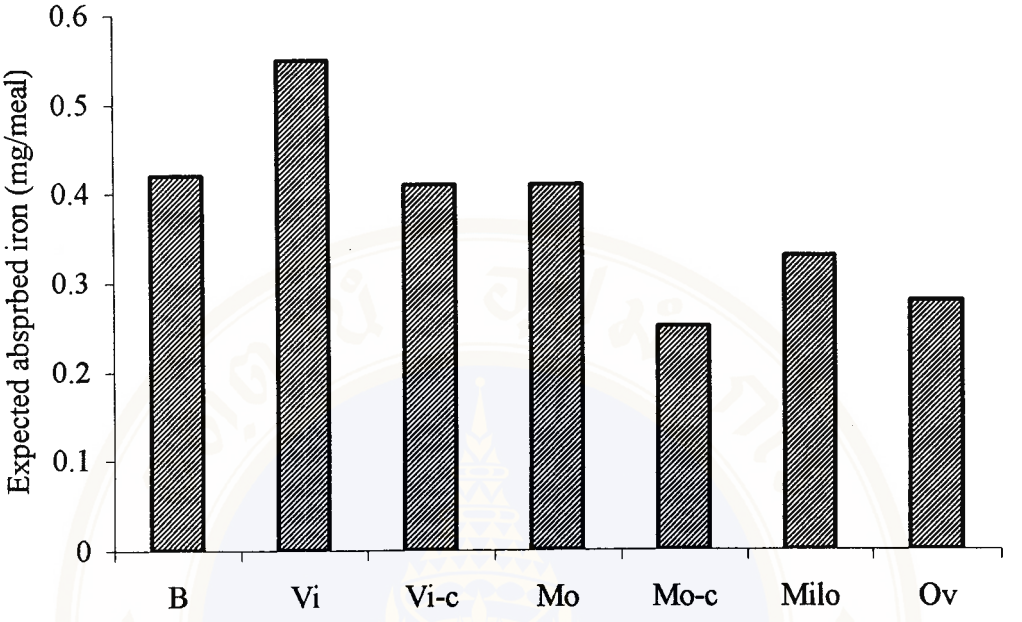

Figure 6. Estimation of the amount of iron expected to be absorbed from the more complex steamed rice meals with supplement food.

As shown in Table 18(a) and 18(b), drinking orange juice with boiled rice and steamed rice breakfasts improved food iron availability at a significant level of $p < 0.05$ when compared with other drinks. Drinking tea with breakfast remarkably decreased iron absorption ($p < 0.001$). Coffee, although contain considerable amount of tannin, its effect was milder. Drinking milk with breakfast showed unnoticeable effect when compared with having breakfast alone, no significant difference was observed.

Table 18(c) shows the effects of different supplement foods on food iron availability from some free choice breakfast meals in group 3. It has been shown that

Vitamax, a vanilla taste cereal, significantly improved iron absorption at $p < 0.05$. Other supplement food with chocolate showed some degrees of inhibitory effects although significant differences were not seen.

TABLE 18. Discrepancy in p-values of the difference among the means of absorbed iron in 3 different groups of breakfast.

a) Boiled rice meals with drinks

	+ O	+ M	+ C	+ T
B	0.002	0.240	0.754	0.000
O		0.001	0.020	0.000
M			0.721	0.000
C				0.001

b) Steamed rice meals with drinks

	+ O	+ M	+ C	+ T
B	0.038	0.596	0.754	0.000
O		0.085	0.007	0.000
M			0.019	0.000
C				0.004

c) Steamed rice meals with supplement foods

	+ Vi	+ Vi-c	+ Mo	+ Mo-c	+ Mi	+ Ov
B	0.049	1.000	0.868	0.031	0.237	0.056
Vi		0.020	0.022	0.001	0.002	0.001
Vi-c			0.819	0.022	0.110	0.012
Mo				0.040	0.245	0.044
Mo-c					0.171	0.618
Mi						0.262

CHAPTER V

DISCUSSION

A drink or supplement food taken together with meals may affect the availability of dietary nonheme iron by the contents of factor enhancing or inhibiting the absorption of iron. In this study the effect of drinks and supplement foods on the ionizability of nonheme food iron in breakfast meals was investigated.

The most marked increase of the ionizability of iron was obtained with orange juice, the amount of estimated absorbed iron increased almost by 3 times. This finding agrees with the results in previous studies (77,79,80). The promoting effect of orange juice is probably one of the most effective way of increasing the bioavailability of iron in a meal.

In this study no effect of milk on the ionizability of nonheme iron from breakfast meals was seen. No significant change in the estimated absorbed iron was observed. This finding is incompatible with results previously reported (7,110,112). Differences in the methods of measuring may explain the differences in the result. The in vitro method used in this study can demonstrate the effect of milk only at the intestinal site while the in vitro method can measured the iron absorbed into the circulation from the mucosal cell.

The decrease in the percentage of iron availability was seen when coffee was added to the meals. The percentage reduction ranged between 23 to 60%. This inhibition can be explained by the present of tannin in coffee. Coffee also contains chorogenic acid, which responsible for the inhibitory effect as well (122). However, the effect of coffee was weaker than tea, which had a marked reduction effect (85 to 88%). Considering the high consumption of coffee and tea together with or immediately after meals, it seems important that further studies are needded on role of long term consumption of coffee and tea in relation to iron nutrition.

Supplement foods taken with meals had interesting effect since it is in general expected that they are good for health and sometimes used as food for the elderly and children. The increase in the percentage of the ionizability by 27%, which reflected the increase in the amount of absorbed iron from taking a vanilla taste cereal with breakfast meals, was quite unexpectes. We cannot identify factors which could responsible for the promotive effect since its ingredients are not clearly clarified. However, no enhance effect was observed from the other brand of a vanilla teste cereal. The inhibitive effect of chocolate taste cereal, milo and ovaltine were well seen as they contain rather high contents of tannin.

The first 2 breakfast meals had rather low contents of iron with a median value of approximately 1.0 mg per meal. The net effect of these drinks on iron nutrition not only related to the content of enhancing or inhibiting factors but also their contents of iron. It was found that the expected amount of iron to be absorbed from drinks taken with a variety of breakfast meals were extremely low which would not be sufficient for the high risk groups of populations to maintain their iron balance. The extra iron intake can be of importance in contributing to dietary iron contents. The groups 3

breakfast taken with supplement foods could provide satisfactory amounts of absorbed iron except those taken with chocolate taste cereal, milo and ovaltine. This study indicate that iron nutrition in a population cannot only be based on the daily dietary intake of iron but must also include the bioavailability of iron in the frequently consumed meals. The choice of drinks and supplement foods could be markedly affect the amounts of nonheme iron to be absorbed from the meals.

CHAPTER VI

CONCLUSION

Nonheme food iron ionizability for estimating the amounts of iron to absorbed was determined in breakfast meals with and without different drinks and supplement foods. Thirty-four menus of breakfast meals were studied. They were divided into 3 groups 1) nine menus of boiled rice meals, 2) ten menus of simple steamed rice meals, 3) fifteen menus of more complex steamed rice meals. In group 1 and 2, four kinds of drink which are orange juice, milk, coffee and tea were tested separately. Six brands of the supplement food which are Vitamax, Vitamax- chocolate, Monie Gold, Monie Gold- chocolate, milo and ovaltine were tested in group 3 breakfast. The ionizable iron was determined using by in vitro ^{59}Fe radiometric method which based on the simulation of gastrointestinal digestion and absorption followed by a measurement of soluble iron. General characteristics in 3 groups of test meals were analysed by nonparametric Kruskal Wallis test. It was shown that the iron content, iron density, phosphorus and phytate contents among the three test meals were significant different at $p < 0.001$ except for the caloric intake. In the first and second breakfast meals, it was shown that orange juice improved food iron availability approximately by 2.5 time, milk showed almost no effect (2%, 5%), coffee and tea decreased iron absorption (23%, 85%) and (60%, 88%) respectively. Group 3 breakfasts with Vitamax-

chocolate, Monie Gold- chocolate, milo and ovaltine decreased the percentage of food iron ionizability by 37%, 64%, 54%, 62% respectively. One vanilla taste cereal improved the percent ionizable iron by 27% and another one show no significant effect. Statistical analysis using Mann Whitney test showed that the amount of estimated absorption iron among the 3 groups of breakfast was different at a significant level of 0.05. There was no significant different between the first and the second group breakfast, but breakfast with supplement food was significantly higher than the first 2 groups at $p< 0.05$. These findings indicate that eating behaviors and choice of drinks and supplement food can affect iron nutrition.

REFERENCES

1. Cammack R, Wrigglesworth JM & Baum H : Iron – dependent enzymes in mammalian systems. In : Iron transport and storage, eds. P.Ponka, H.M., Schulman & RC Woodworth. Boca Raton : CRC Press ; 1990. p.17 – 39.
2. Crichton RR : Inorganic biochemistry of iron metabolism. Chichester : Ellis Horwood ; 1991. p.7 – 263.
3. Bothwell TH, Charlton RW, Cook JD & Finch CA, eds. Iron metabolism in man. Oxford : Blackwell Scientific Publications, 1979.
4. Cook JD, Monsen ER. Food iron adsorption in human subjects II . The effect of EDTA on absorption of dietary nonheme iron. Am J Clin Nutr 1976 ; 29 : 614.
5. Cook JD, Monsen ER Food iron absorption in human subjects III . Comparison of the effect of animal protein on nonheme iron absorption . Am J Clin Nutr 1976 ; 29 : 859 .
6. Tuntawiroon M, Sritongkul N, Brune M, et al . Dose - dependent inhibitory effect of phenolic compounds in foods on non - heme iron absorption in man . Am J Clin Nutr 1991 ; 53 : 554 - 557 .
7. Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander L. Calcium : Effect of different amounts on non - heme and heme - iron absorption in humans. Am J Clin Nutr 1991 ; 53 : 112 - 119.

8. Cook. JD, Layris'se M, Martinez - Torres C, Walker R, Monsen E & Finch CA. Food iron absorption measured by an extrinsic tag. J Clin Invest 1972 ; 51 : 805 - 815.

9. Hallberg L & Bjorn - Rasmussen E. Determination of iron absorption from whole diet. A new two - pool model using two radioiron isotopes given as haem and non - haem iron. Scandinav. J Haematol. 1972 ; 193 - 197.

10. Hallberg L. The pool concept in food iron absorption and some of its implications. Proc. Nutr. Soc. 1974 ; 33, 285.

11. Rao BSM, Prabhavathi T, An in vitro method for predicting the bioavailability of iron from food. Am J Clin Nutr 1978 ; 31 : 169 - 175.

12. Miller DD, Schricker BR, Rasmussen RR, Van Campen D. An in - vitro method for estimation of iron availability from meals. Am J Clin Nutr 1981 ; 34 : 2248 - 2256.

13. Chidambaram MV, Reddy MB, Thompson JL, Bates GW. In vitro studies of iron bioavailability. Biol. Trace Elem Res 1989 ; 73 : 1245 - 1248.

14. Schricker BR, Miller DD., Rasmussen RR, Van Campen D. A comparison of in vivo and in vitro methods for determining availability of iron from meals. Am J Clin Nutr 1980 ; 37 : 2257 - 2263.

15. Forbes AL, Adams CE, Arnaud MJ et.al. Comparison of in vitro, animal and clinical determination of iron bioavailability : International Nutritional Anemia Consultative Group Task Force report on iron bioavailability. Am J Clin Nutr 1989 ; 49 : 225 – 238.

16. Whiltaker, P, Fox MRS, Forbes AL. In vitro prediction of iron bioavailability for food fortitication. Nutr Rep Int 1989 ; 39 : 1205 - 1215.

17. Kane AP and Miller DD. In vitro estimation of the effects of selected protein on iron availability. Am J Clin Nutr 1984 ; 39 : 393 - 401.

18. Harper HA, Rodwell VW and Mayes PA. Review of Physiological Chemistry (17 th ed). Los Altos, Calif : Lange; 1979. p.585.

19. Beutler E and Fairbanks VF. The effects of iron deficiency. In : Iron in Biochemistry and Medicine 2 nd dn (eds A. Jacobs and M. Worwood), Academic Press, London; 1980. p.393.

20. Dallman PR. Biochemical basis for the manifestations of iron deficiency. Annu. Rev. Nutr. 1986 ; 6 : 13 – 40.

21. Finch CA and Huebers H. Perspectives in iron metabolism. New Engl. J Med.1982 ; 306 : 1520 – 1528.

22. Huebers HA and Finch CA. The physiology of transferrin and transferrin receptors. Physiol. Reviews 1987 ; 67 : 520 – 582.

23. Bothwell TH & Finch CA. Iron losses in man. In : Occurrence, Causes and Prevention of Nutritional Anaemias. Symposia of the Swedish Nutrition Foundation. VI. Almqvist and Wiksell, Uppsala ; 1968. p. 104 – 114.

24. Green R, Charlton RW, Seftel H, Bothwell T, Mayet F, Adams B, et al. Body iron excretion in man. A collaborative study. Amer. J. Med. 1968 ; 45 : 336 – 353.

25. Hallberg L, Hogdahl AM, Nilsson L and Rybo G. Menstrual blood loss – a population study. Variation at different ages and attempts to define normality. Acta. Obstet. Gynaecol. Scand. 1966 ; 45 : 320 – 351.

26. Hallberg L & Nilsson L. Constancy of individual menstrual blood loss. Acta. Obstet. Gynaecol. Scand. 1964 ; 43 : 352 – 359.

27. Hallberg L & Rossander – Hulthen L. Iron requirements in menstruating woman. Am J Clin Nutr 1991 ; 54 : 1047 – 1058.

28. Nilsson L and Solvell L. Clinical studies on oral contraceptives – a randomized, double blind, crossover study of four different preparation. Acta. Obstet. Gynaecol. Scand. (Suppl) 1967 ; 46 : 1 – 31.

29. Guillebaud J, Bonnar J, Morehead J & Matthews A. Menstrual blood – loss with intrauterine devices. Lancet 1976 ; 1 : 387 – 390.

30. Baynes RD & Bothwell TH. Iron deficiency. Annu Rev Nutr 1990 ; 10 : 133 – 148.

31. Hallberg L. Iron balance in pregnancy. In : Vitamins and Minerals in Pregnancy and Lactation (ed. H. Berger), Nestle' Ltd, Raven Vevey New York, p.115.

32. Beveridge BR, Bannerman RM, Evanson JM and Witts LJ. Hypochromic anaemia. A retrospective study and follow – up of 378 in – patients. Q. J. Med.1965 ; 34 : 145 – 161.

33. Skikne BS. Current concepts in iron deficiency anemia. Food Rev. Int. 1988 ; 4 : 137 – 173.

34. Layrisse M and Roche M. The relationship between anemia and hookworm infection. Am J Hyg 1964 ; 79 : 279 – 301.

35. Parry DJ & Wood PHN. Relationship between aspirin taking and gastroduodenal haemorrhage. Gut 1967 ; 8 : 301.

36. Fleming JL, Ahlquist DA, McGill DB, Zinsmeister AR, Ellefson RD and Schwartz S. Influence of aspirin and ethanol on fecal blood levels as determined by using the Hemo Quant assay. Mayo Clin Proc. 1987 ; 62 : 159 – 163.

37. Finch CA, Cook JD, Labbe RF and Culala M. Effect of blood donation on iron stores as evaluated by serum ferritin. Blood 1977 ; 50 : 441- 447.

38. Josephs HW. Hypochromic microcytic anemia of infancy : iron depletion as a factor. Pediatrics 1956 ; 18 : 959 – 978.

39. Kaldor I. Storage iron in the fetus. Australas. Ann Med 1955 ; 4 : 277- 278.

40. Widdowson EM & Spray CM. Chemical development in utero. Arch. Dis. Child.1951 ; 26 : 205 - 214.

41. Scip M and Halvorson S. Erythrocyte production and iron stores in premature infants during the first months of life : the anemia of prematurity – etiology, pathogenesis, iron requirement. Acta Paediat 1956 ; 45 : 600 – 617.

42. Susan J. Fairweather – Tait. Iron requirements and prevalence of iron deficiency in adolescents. An overview. In : Iron Nutrition in Health and Disease, edited by Leif Hallberg and Nils – Georg Asp. John Libbey & Company Ltd ; 1996. p. 137 – 148.

43. FAO / WHO Joint Expert Consultation Report. Requirements of Vitamin A, Iron, Folate and Vitamin B_{12}. FAO Food and Nutrition Series 23, Food and Agriculture Organization, Rome ; 1988.

44. Wretlind A. Food Iron Supply, In : Iron deficiency, edited by. Hallberg L, Harwerth HG and Vannolti A. Academic Press, London and New York ; 1970. P. 39 – 70.

45. United States Department of Health, Education and Welfare Publication No. 72 – 8131 : Ten States Nutrition Survey ; 1972.

46. Wretlind A. The supply of food iron. In : Occurrence, Causes and Prevention of Nutritional Anaemias. Symposia of the Swedish Nutrition Foundation. VI. Ed. G. Blix. Almqvist & Wiksell, Stockholm ; 1968. P. 73 – 91.

47. Hazell T. Minerals in foods : Dietary source, chemical forms, interaction, bioavailability. Wld Rev Nutr Diet. 1985 ; 46 : 1 – 123.

48. Layrisse M, Cook JD, Martinez C, Roche M, Kuhn IN, Walker RB, et al. Food iron absorption : a comparison of vegetable and animal foods. Blood. 1969 ; 33 : 430 – 443.

49. Bothwell TH, Baynes RD, MacFarlane BJ and MacPhail AP. Nutritional iron requirements and food iron absorption J Int. Med. 1989 ; 226 : 357 – 365.

50. Wheby MS. Site of iron absorption in man. Scandinav J Haemat.1970 ; 7 : 56 – 62.

51. Manis JG & Schachter D. Active transport of iron by intestine : features of the two – step mechanism. Amer J Physiol. 1962 ; 203 : 73 – 80.

52. Wheby MS & Crosby WH. The gastrointestinal tract and iron absorption. Blood 1963 ; 22 : 416 – 428.

53. Wheby MS, Sultle GE & Ford K.T. Intestinal absorption of hemoglobin iron. Gastroenterology 1970 ; 58 : 647 – 654.

54. Wheby MS, Jones LG & Crosby WH. Studies on iron absorption. Intestinal regulatory mechanisms. J Clin Invest 1964 ; 43 : 1433 – 1442.

55. Grasbeck R, Majuri R, Kouvonen I and Tenhunen R. Spectral and other studies on the intestinal haem receptor of the pig. Biochem Biophys Acta. 1982 ; 700 : 137 – 142.

56. Raffin SB, Woo CH, Roost KT, Price DC and Schmid R. Intestinal absorption of hemoglobin iron – heme cleavage by mucosal heme oxygenase. J Clin Invest 1974 ; 54 : 1344 – 1352.

57. Layrisse M, Martinez – Torres C, Mendez – Castellano H. Taylor P, Fossi M, Lopes de Blanco M, et al. Relationship between iron bioavailability from diets and the prevalance of iron deficiency. Food Nutr Bull 1990 ; 12 : 301 – 309.

58. Cook JD, Morck TA, Skikn BS and Lynch SR. The importance of animal products in human iron nutrition. In : Animal Products in Human Nutrition (eds. D.C. Beitz and AG. Hansen), Academic Press, New York ; 1982. p. 321.

59. Bezwoda WR, Bothwell TH. Charlton RW, et al. The relative dietary importance of heme and non – heme iron. S. Afr. Med. J. 1983 ; 64 : 552 – 556.

60. Hallberg L, Bjorn – Rasmussen E, Howard L and Rossander L. Dietary heme iron absorption. A discussion of possible mechanisms for the absorption – promoting effect of meat and for the regulation of iron absorption. Scand J Gastroenterol 1979 ; 14 : 769 – 779.

61. Martinez – Torres C & Layrisse M. Iron absorption from veal muscle. Am J Clin Nutr 1971 ; 24 : 531 – 540.

62. Heinrich H, Gabbe E & Kugler G. $^{59}Fe^{2+}$ and hemoglobin ^{59}Fe absorption in human being do not require gastric juice or intrinsic factor. Biochem. Med. 1987 ; 5 : 472 – 482.

63. Gabbe EE, Heinrich HG, Bruggemann J and Pfau AA. Iron absorption from hemoglobin (stable oxidation product of hemoglobin) in relation to the dose in subjects with normal and depleted iron stores. Nutr Metab. 1979 ; 23 : 17- 25.

64. Wheby MS and Spyker DA. Hemoglobin iron absorption kinetic in the iron deficient dog. Am J Clin Nutr 1981 ; 34 : 1686 – 1693.

65. Conrad ME, Cortell S, Williams HL and Foy AL. Polymerization and intraluminal factors in the absorption of hemoglobin iron. J Lab Clin Med. 1966 ; 68 : 659 – 668.

66. Schricker BR & Miller DD. Effects of cooking and chemical treatment on heme and non – heme iron in meat. J. Food. Sci. 1983 ; 48 : 1340 – 1349.

67. Martinez – Torres C Leeb I, Taylor P, Ramirez J, del Valle Camacho M & Layrisse M. Heme, ferritin and vegetable iron absorption in humans from meals denatured of heme iron during the cooking of beef. J Nutr 1986 ; 116 : 1720 – 1725.

68. Bezwoda W, Charlton R, Bothwell T, Torrance J and Mayet F. The importance of gastric hydrochloric acid in the absorption of non – heme food iron. J Lab Clin Med. 1978 ; 92 : 108 – 116.

69. Jacobs P, Charlton RW and Bothwell TH. The influence of gastric factors on the absorption of iron salts. S. Afr. J. Med. Sci. 1968 ; 33 : 53 – 57.

70. Wollenberg P & Rummel W. Dependence of intestinal iron absorption on the valency state of iron. Arch. Pharmacol 1987 ; 336 : 578 – 582.

71. Zempsky WT, Rosenstien BJ, Carroll JA and Oski FA. Effect of pancreatic enzyme supplements on iron absorption. Am J Dis Child. 1989 ; 143 : 969 – 972.

72. Monsen ER and Cook JD. Food iron absorption in human subjects. V. Effects of the major dietary constituents of semisynthetic meal. Am J Clin Nutr 1979 ; 32 : 804 – 808.

73. Moore CV, Dubach R, Minnich V and Roberts HK. Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. J Clin Invest. 1944 ; 23 : 755.

74. Hahn PF, Jones E, Lower RC, Meneely GR and Peacock W. The relative absorption and utilization of ferrous and ferric iron in anemia as determined with the radioactive isotope. Am J Physiol. 1945 ; 143 : 191.

75. Brise H & Hallberg L. Absorbability of different iron compounds. Acta. Med. Scand. (Suppl) 1962 ; 17 : 32 – 44.

76. Layrisse M, Martinez – Torres C & Roche M. The effect of interaction of various foods on iron absorption. Am J Clin Nutr 1968 ; 21 : 1175 – 83.

77. Lynch SR and Cook JD. Interaction of Vitamin C and iron. Ann. NY. Acad Sci.1980 ; 335 : 32 – 44.

78. Cook JD and Monsen ER. Vitamin C, the common cold and iron absorption. Am J Clin Nutr 1977 ; 30 : 235 – 241.

79. Hallberg L, Brune M & Rossander L. Effect of ascorbic acid on iron absorption from different types of meals. Ann Nutr Appl Nutr 1986 ; 40 A: 97 – 113.

80. Hallberg L, Brune M & Rossander L. Iron absorption in man : ascorbic acid and dose – dependent inhibition by phytate. Am J Clin Nutr 1989 ; 49 : 140 – 144.

81. Tuntawiroon M, Sritongkul N, Rossander – Hulthen L, Hallberg L & Brune M. Rice and iron absorption in man. Eur J Clin Nutr 1990 ; 44 : 489 – 479.

82. Siegenberg D, Baynes RD & Bothwell TH. Ascorbic acid prevents the dose – dependent inhibitory effect of polyphenols and phytate on non – heme iron absorption. Am J Clin Nutr 1991 ; 53 : 537 – 541.

83. Hallberg L, Rossander L, Persson H & Svahn E. Deleterious effect of prolonged warming of meals on ascorbic acid content and iron absorption. Am J Clin Nutr 1984 ; 39 : 577 – 581.

84. Lynch SR, Hurrell RF, Dassenko SA and Cook JD. The effect of dietary proteins on iron bioavailability in man. Adv. Exp. Med. Biol. 1989 ; 249 : 117 – 132.

85. Christensen JM, Ghannam M and Ayres JW. Effect of divalent amino acids on iron absorption. J Pharm. Sci. 1984 ; 73 : 1245 – 1248.

86. Martinez – Torres C, Romano E & Layrisse M. Effect of cysteine on iron absorption in man. Am J Clin Nutr. 1981 ; 34 : 322 – 327.

87. Taylor PG, Martinez – Torres C, Romano EL & Layrisse M. The effect of cysteine – containing peptides released during meat digestion on iron absorption in humans. Am J Clin Nutr. 1986 ; 43 : 68 – 71.

88. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA and Cook JD. Iron absorption in humans : bovine serum albumin compared with beef muscle and egg white. Am J Clin Nutr. 1988 ; 47 : 102 – 107.

89. Derman DP, Bothwell TH, Torrance JD, et al. Iron absorption from maize (Zeamays) and sorghum (Sorghum valgar) beer. Brit Med J 1980 ; 43 : 271 – 279.

90. Gillooly M, Bothwell TH, Torrance JD, et al. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr 1983 ; 49 : 331 – 342.

91. Ballot D, Baynes RD, Bothwell TH, et al. The effect of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr. 1987 ; 57 : 331 – 343.

92. Bjorn – Rasmussen E. Iron absorption from wheat bread. Influence of various amounts of bran. Nutr Metab 1974 ; 16 : 101 – 110.

93. Rossander – Hulthen L, Gleerup A AND Hallberg L. Inhibitory effect of oat products on nonhaem iron absorption in man. Eur J Clin Nutr. 1990 ; 44 : 783 – 791.

94. Hallberg L, Rossander L and Skanberg AB. Phytates and the inhibitory effect of bran on iron absorption in man. Am J Clin Nutr. 1987 (b) ; 45 : 988 – 996.

95. Morris ER and Ellis R. Phytate, wheat, bran and bioavailability of dietary iron. In: Nutritional Bioavailability of Iron. (ACS Symposium Series 203), American Chemical Society, Washington DC ; 1982. p. 121.

96. Sanderson GW, Ranadive AS, Eisenberg LS, Farrell FJ, Simons R, Manley CH, et al. Contribution of polyphenolic compounds to the taste of tea. In : Phenolic, Sulfur and Nitrogen Compounds in Food Flavors. Charelambous G & Katz I, EDS. ACS Symp. Ser. No 26. Am Chem Soc.Washington DC ; 1976.

97. Clifford MN, Wight J. The measurement of feruloquinic acid and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans. J Sci Food Agric 1976 ; 27 : 73 – 84.

98. Singleton VL, Noble AC. Wine flavor and phenolic substances. In : Phenolic, Sulfur and Nitrogen compounds in food flavors. Charalambous G and Katz I. Eds Am Chem Soc Symp 1976 ; 26 : 47 – 70.

99. Desphande SS, Cheryan M. Determination of phenolic compounds of dry beans using vanillin, reduce and precipitation assays. J Food. Sci 1987 ; 52 : 332 – 334.

100. Griffith DW. The polyphenolic content and enzyme inhibitory activity of test as from bean (Vicia faba) and pea (Pisum spp) varieties. J Sci Food Agric 1981 ; 32: 797.

101. Davis KR. Effect of processing on composition and Tertrahymena relative nutritive value of green and yellow pea, lentils and white pea beans. Cer Chem 1981 ; 58 : 454.

102. Narasinga Rao BS, Prabhavathi T. Tannin content of foods commonly consumed in India and its influence on ionisable iron. J Sci Food Agric 1982 ; 33 : 89.

103. Earp CF, Akingbala JO, Ring SH, Rooney LW. Evaluation of several methods to determine tannins in sorghums with varying kernel characteristics. Cereal Chem 1981 ; 58 : 234.

104. Mahanso H, Butler LG, Carlson DM. Dietary tannins and salivary proline – rich proteins : Interactions, induction and defense mechanisms. Ann Rev Nutr 1987 ; 7 : 423 – 40.

105. Disler PB, Lynch SR, Charlton RW,et al. The effect of tea on iron absorption. Gut 1975 ; 16 : 193 – 200.

106. Hallberg L and Rossander L. Effect of different drinks on the absorption of non – heme iron from composite meals. Hum Nutr. Appl Nutr. 1982 ; 36 : 116 – 123.

107. Morck TA, Lynch SR and Cook JD. Inhibition of food iron absorption by coffee. Am J Clin Nutr 1983 ; 37 : 416 – 420.

108. Monsen ER & Cook JD. Food iron absorption in human subjects. IV. The effect of calcium and phosphate salts on the absorption of non – heme iron. Am J Clin Nutr 1976 ; 29 : 1142 – 1148.

109. Cook JD, Dassenko SA and Whiltaker P. Calcium supplementation : Effect on iron absorption. Am J Clin Nutr 1991 ; 53 : 106 – 111.

110. Gleerup A, Rossander – Hulthen L & Hallberg L. Duration of the inhibitory effect of calcium on non – heme iron absorption in man. Eur J Clin Nutr 1993 ; 47 : 875 – 879.

111. Hallberg L, Rossander – Hulthen L, Brune M & Greerup A. Inhibition of haem – iron absorption in man by calcium. Brit J Nutr 1993 ; 69 : 533 – 540.

112. Layrisse M, Martinez – Torres C, Cook JD, Walker R and Finch CA. Iron fortification of food : its measurement by the extrinsic tag method. Blood 1973 ; 41 : 333 – 352.

113. Hallberg L. Food iron absorption. In : Methods in Hematology :Ir (ed. JD. Cook) Churchill Livingstone, New York ; 1980. p. 116.

114. Hallberg L. Bioavailable nutrient density : a new concept applied in the interpretation of food iron absorption data. Am J Clin Nutr 1981 ; 34 : 2242 – 2247.

115. Magnusson B, Bjorn – Rasmussen E, Hallberg L and Rossander L. Iron absorption in relation to iron status. Model propose to express results to food iron absorption measurement. Scand J Haematol 1981 ; 27 : 201 – 208.

116. Chidambaram MV, Reddy MB, Thompson JL and Bates GW. In vitro studies of iron bioavailability : probing the concentration and oxidation – reduction reactivity of pinto bean iron with ferrous chromogens. Biol Trace Elem Res 1989 ; 19 : 25 – 39.

117. Schricker BR, Miller DD ,Rasmussen RR & Van Campen D. A comparison of in vivo and in vitro methods for determining availability of iron from meals. Am J Clin Nutr 1981 ; 34 : 2257 – 2263.

118. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA & Cook JD. Iron absorption in humans as influenced by bovine milk protein. Am J Clin Nutr 1989 ; 49 : 546 – 552.

119. Sritongkul N, Tuntawiroon M, an in vitro method for estimation of available dietary iron. Siriraj Scientific Annual Meeting (34 th) March ;1994.

120. Official Methods of Analysis of the Association of official Agricultural Chemists (10 th ed.) Washington DC : Association of official Agricultural Chemists ; 1965. p.192.

121. Harland EF and Oberleas D. Anion exchange method for determination of phytate in food : Collaborative study. J Assoc of Anal Chem 1986 ; 69 : 667 – 670.

122. Brune M, Rossander L. and Hallberg L. Iron absorption and phenolic compounds : importance of different phenolic structure. Eur. J Clin Nutr 1989 ; 43 : 547 – 558.

APPENDIX A

Determination of ionizable iron

Principle

An inorganic radioiron tracer (^{59}Fe) is added to a homogenized food sample. After a two-stage (pepsin and pancreatin) digestion, the ionizable iron was determined by using bathophenanthroline solution. The bathophenanthroline reactive iron was extracted with isoamyl alcohol. The ^{59}Fe activity in isoamyl alcohol extracted was calculated as the percentage of ionizable iron against total amount of radioiron added to homogenizied food sample.

Procedure

1. Weigh 20 gm of homogenized food sample into 125 ml Erlenmeyer flask.
2. Add 20 ml 0.17 N HCl, 5 ml 1% Pepsin and 500 µl ^{59}Fe.
3. Mix and incubate in a waterbath, 37°c for 60 minutes.
4. After incubation, weigh out the digestion mixture 1 gm into counting test tube, it's activity represent the total amount of radioiron added to food sample. Another 4 gm was weighed into 15 ml test tube for the next incubation.

5. Add 3 ml acetate buffer and 1 ml 1% pancreatin in 0.1 M $NaHCO_3$. Mix and then divide into 2 sets, one without adjusting pH and the other adjust pH to 6.8 by using NaOH (conc.).
6. Mix and incubate 37°c for 2 hours.
7. After 2 hours add 1.0 ml chloroform and mix. The tube is then centrifuged 2000 rpm, at room temperature for 10 minutes.
8. Pipette 1ml supernatant into counting test tube. This represents the half dilution counts. Another triplicate of 0.5 ml was pipetted into 6 ml screw cap vials.
9. Add 1ml bathophenanthroline solution into screw cap vials. Mix and then shake for 90 minutes at room temperature.
10. Add 1 ml isoamyl alcohol after the shaken period, mix and centrifuge 2000 rpm at room temperature for 5 minutes.
11. The upper isoamyl alcohol layer is pipetted off 1 ml to counting test tube for measuring ^{59}Fe activity as the ionizable iron.
12. Radioactivity in the total, half dilution and ionizable iron tubes were measured in a LKB Wallac Gamma counter.
13. Calculate the percentage of ionizable iron by using the following equation.

$$\text{Ionizable iron } (^{59}Fe)\% = \frac{\text{cpm}^{59}\text{Fe/ml isoamyl alcohol extract x 5.6* x 100}}{\text{cpm}^{59}\text{Fe/g of meal (Total counts)}}$$

*Sample dilution factor

Figure 1A. Homogenized food sample in Erlenmeyer flask.

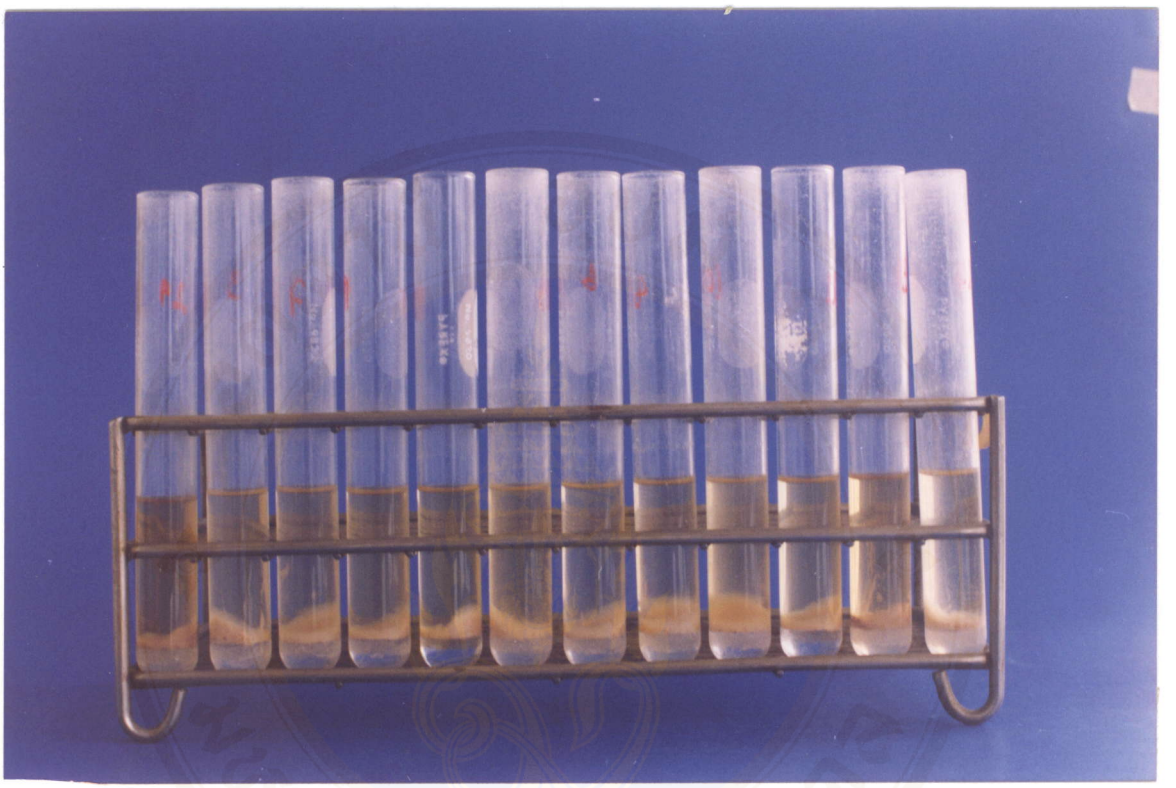

Figure 2A. After two-stage (Pepsin-Pancreatin) digestion of homogenized test meal, added chloroform will settle down the lipid from digestion mixture and separate soluble iron in the supernatant.

Figure 3A. Triplicate of 0.5 ml supernatant of digestion mixture was pipetted into 6 ml screw cap vials.

Figure 4A. Bathophenanthroline solution was added to the supernatant of digestion mixture in a screw cap vial. Mix and then shake for 90 minutes at room temperature.

Figure 5A. The upper isoamyl alcohol was the extracted bathphenanthroline reactive iron from the digestion mixture.

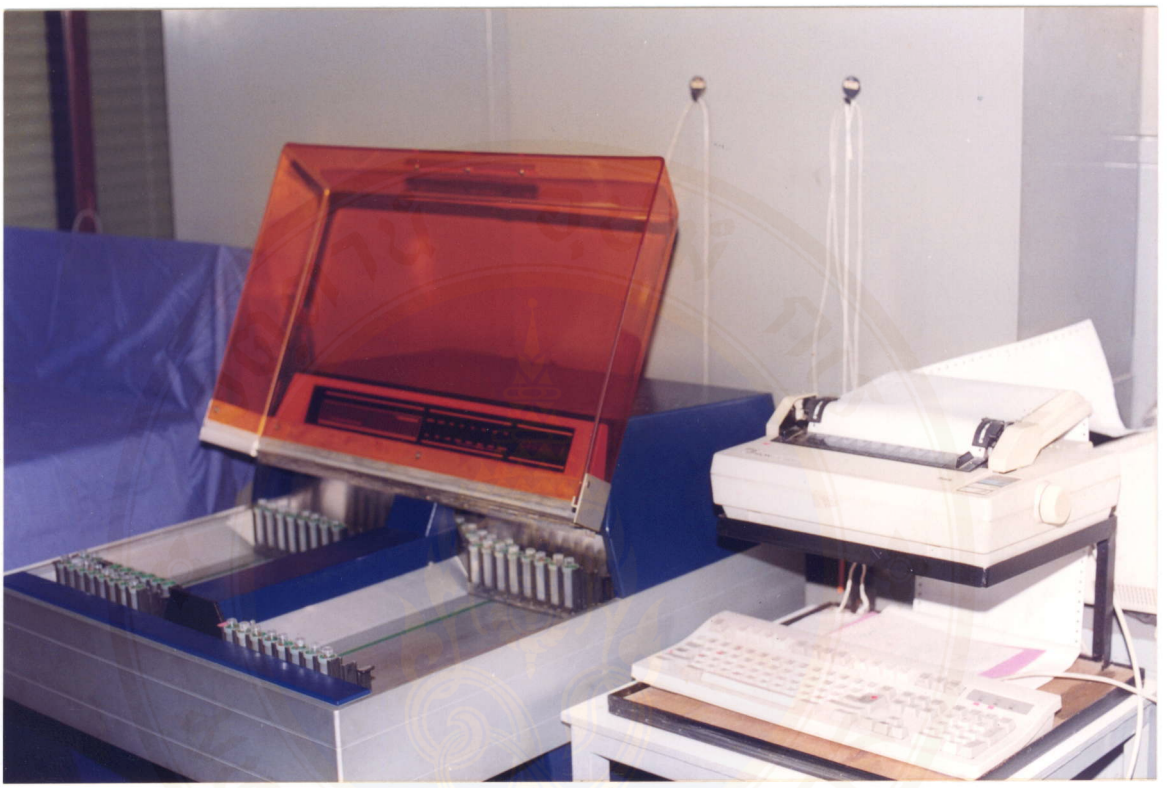

Figure 6A. Radioactive measurement was counted in a LKB Wallac Gamma counter.

APPENDIX B

Determination of total iron

Principle

The organic material in food sample was ashed by the mixture of acids. Iron content of the ash was estimated by developing colour with bathophenanthroline sulphonate.

Procedure

1. Weigh 10 gm homogenized food sample into a 100 ml Kjeldahl flask and add 3 glass beads.
2. Add 2.5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid. Digest with low heat and gradually increase the heat until it become dark brown colour. Allow it to cool.
3. Repeat step 2 again until the solution become orange. Allow it to cool.
4. Add 2.5 ml concentrated nitric acid. Heat until all the orange colour changes to yellow.
5. Once the material has become light yellow, allow the sample to cool and add 2 ml of 30% hydrogen peroxide (H_2O_2). Heat until all the hydrogen peroxide fumes

have evolved, and allow it to cool. Repeat this step if the sample is not water white.

6. Transfer the sample quantitatively without glass beads to 50 ml volumetric flask, with three to four times washing of iron free water using approximately 5 ml for each wash.
7. Add 1 drop of 1% paranitrophenol to the volumetric flask and begin adding concentrated ammonium hydroxide slowly until the sample just turns yellow.
8. Allow the flask to cool and make up to volume (50 ml) with iron free water.
9. Include with each analysis an acid blank containing approximately the same volume of acid as was used for the digestion procedure. However, the total volume of acid in this blank should not exceed 15 ml. The acid blank should otherwise be handled in the same manner as the unknown sample.
10. Prepare solutions for colorimetric analysis in iron-free 10 ml glass tubes by placing into each tube 1 ml sodium acetate buffer, 5 ml of sample volume or iron standard with iron free water and 0.2 ml bathophenanthroline sulphonate.
11. The sample were allowed to stand for at least 15 minutes before the measurement of optical density at 535 nm.
12. The iron concentration of sample was calculated in μgFe/gm sample.

TABLE 19. Sample volume for colorimetric analysis.

Sample	Acetate Buffer (ml)	DI H_2O (ml)	Iron STD (ml)	Digest Sample (ml)	Chromogen (ml)
Water Blank	1.0	5.0	-	-	0.2
Acid Blank	1.0	-	-	5.0	0.2
Standard Fe					
1 µg	1.0	4.0	1.0	-	0.2
2 µg	1.0	3.0	2.0	-	0.2
3 µg	1.0	2.0	3.0	-	0.2
4 µg	1.0	1.0	4.0	-	0.2
Unknown	1.0	4.0	-	1.0	0.2

Figure 1B. Homogenized food sample in Kjeldahl flask.

Figure 2B. Add 2.5 ml concentrated sulfuric acid and 2.5 ml concentrated nitric acid into the Kjeldahl flask.

Figure 3B. Digest with low heat and gradually increase the heat until it become dark brown colour.

Figure 4B. Add 2 ml of 30 % hydrogen peroxide (H_2O_2) into Kjeldahl flask.

Figure 5B. After added 1 drop of 1% paranitrophenol, slowly add concentrated ammonium hydroxide until the sample turns yellow.

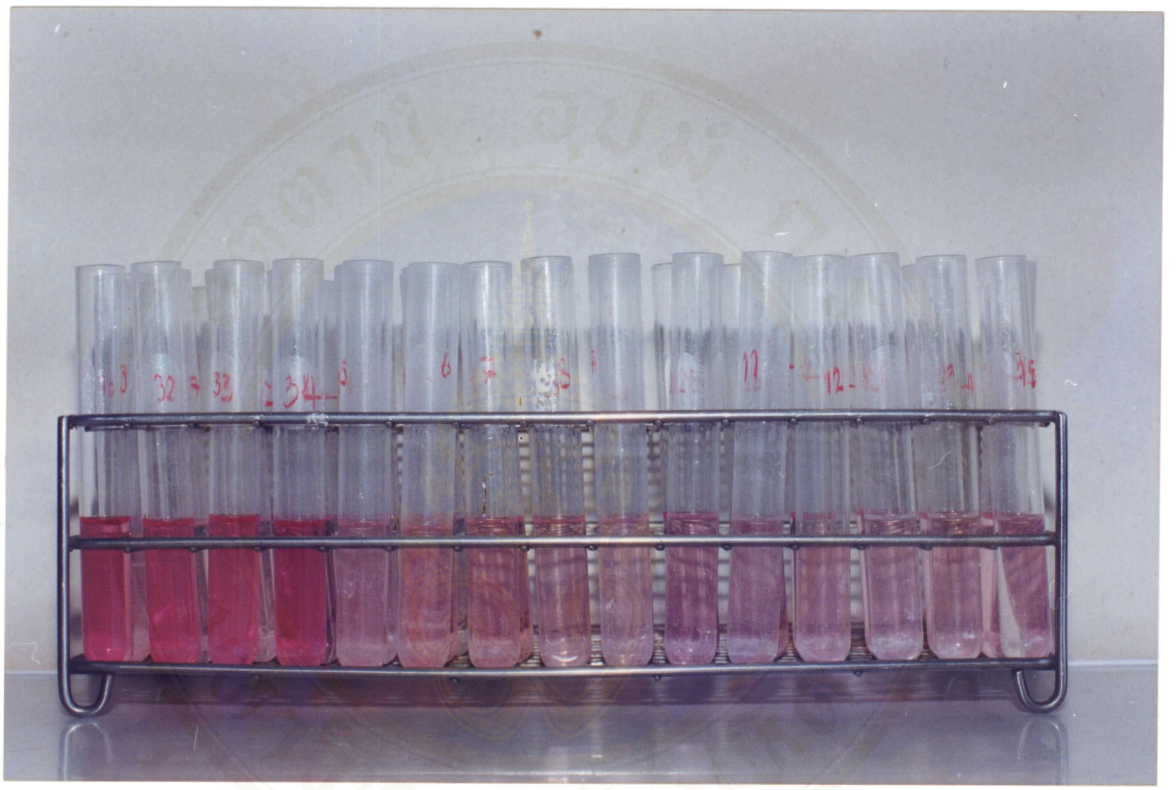

Figure 6B. After added the chromogen solution it becomes red colour which is directly proportional to the amount of iron in the food sample.

Figure 7B. The optical density was read in spectrophotometer at 535 nm.

APPENDIX C

Determination of total phosphorus and phytate

Principle

Phytate is extracted from food samples using 2.4% HCl. The extracted is mixed with an EDTA/NaOH solution and placed on an ion-exchange column. Phytate is eluted with 0.7 M NaCl solution and wet digested with a mixture of concentrated nitric/sulfuric acid to release the phosphorus which is then measured colorimetrically. Amount of phytate in the original sample is calculated as hexaphosphate equivalent.

Procedure

1. Weigh carefully 16 gm of homogenized food sample into a 125 ml Erlenmeyer flask.
2. Add 50 ml 2.4% HCl.
3. Cover flask and shake vigorously at room temperature for 3 hrs.
4. While sample is being shaken to extract the phytate, prepare the column. Use glass barrell columns (0.7 x 15 cm) equipped with a valve.
5. Add 3 ml iron free water to empty, clean, mounted column and then pour a water slurry of 0.5 g resin (anion exchange resin, AGI – X4, 100-200 mesh, chloride form Bio-Rad. Cat No.140-1341) into the column.

6. After the resin bed has formed, wash column with three, 15 ml volumes of 0.7 M NaCl.
7. Then wash column with three, 15 ml volumes of iron free water.
8. Remove sample from shaker and filter through Whatman filter paper (No.541). Sample extract is stable for at least one week, after preparation, if refrigerated.
9. Pipet 4.0 ml of filtrate into a 25 ml volumetric flask. Add 4.0 ml Na_2EDTA-NaOH solution. Bring to 25 ml volume with iron free water.
10. Mix and pour quantitatively onto column; discard eluate.
11. Elute with 15 ml iron free water, discard eluate.
12. Elute with 15 ml of 0.1 M NaCl, discard eluate.
13. Elute with 15 ml of 0.7 M NaCl, collect this fraction in digestion vessel.
14. Add 0.5 ml concentrated sulfuric acid, and 6.0 ml concentrated nitric acid to flask. Add 3 glass beads.

    Note: Before adding the next sample to the column, regenerate the resin by pouring three,15 ml volumes of 0.7 M NaCl and three,15 ml volumes of iron free water through the columns. After a week, discard old resin and replace with fresh resin.
15. Digest under hood on micro-Kjeldahl rack over medium heat until active boiling ceases, and a cloud of thick yellow vapor fills the neck of the flask.
16. Heat contents for 5 minutes more on medium heat, 5 minutes on low heat, then turn off burner.

17. When flask is cool, add 10 ml of iron free water, swirl to dissolve salt, or heat tube on low temperature setting if necessary in order to dissolve salt and continue heat flask on low temperature for 10 min.
18. Permit solution to cool.
19. Transfer solution quantitatively to a 100 ml volumetric flask.
20. Add 4.0 ml molybdate solution, mix well.
21. Add 2.0 ml sulfonic acid reagent, mix well.
22. Make to volume, mix well, let stand 15 min and read absorbance at 640 nm.

Preparation of phosphate standard curve

Reagent

1. Phosphate standard solution.
2. Molybdate solution.
3. Sulfonic acid solution.

Procedure

1. Pipet 0.5, 1.0, 1.5, 3.0, 5.0 and 10.0 ml of standard phosphate solution (80 μg P/ml) into each of three, 100 ml volumetric flasks. Add about 20 ml water. Mix throughly.
2. Add 4 ml molybdate solution. Mix well.
3. Add 2 ml sulfonic acid solution. Mix well.
4. Bring to 100 ml volume with iron free water . Mix well.

5. Equilibrate for at least 15 minutes.

6. Read in a spectrophotometer at 640 nm.

TABLE 20. Standard solution, 80 µg phosphorus/ml.

ml std	µg/P	Absorbance (A)	Conc/A (K)
0.5	40	0.0908	440.53
1.0	80	0.1805	443.21
1.5	120	0.2711	442.64
3.0	240	0.516	465.12
5.0	400	0.852	469.48
10.0	800	1.490	536.91
		Mean K	466.48

Calculation of phytate concentration in food

1. Read Absorbance (A).

2. Multiply Absorbance (A) times "Mean K" to get µg phosphorus per sample or dilution.

(K is derived by dividing concentration of phosphorus in a standard by the absorbance obtained for that standard). It represents the calculated concentration per unit absorbance for each concentration of standard. The "Mean K" obtained

from the several standards is comparable to,but a more precise estimate of the mid point on the best straight line of a standard curve.

3. Multiply by 0.781 to obtain µg phosphorus per gm of sample. (0.781 is dilution factor derived as follows: $\frac{50}{(16x4)}$ where 50 is ml of extraction acid, 16 is the weight of the sample in gm, and 4 is ml of the extractable filtrate placed on the column).
4. Divide by 1000 to get mg of phosphorus per gm of sample.
5. Divide by 0.282 to convert mg of phosphorus per gm of sample to mg of phytate per gm of sample. (The phytate molecule is 28.2% phosphorus).

Calculation

mg phytate / g sample = Mean K x A x 0.781 / (0.282 x 1000)

Determination of total phosphorus

Total phosphorus in the extracted sample was determined by pipetting 4 ml of filtrate in step 8 into digestion vessel, add 11 ml of 0.7 M NaCl and then follow the procedure from step 14 to 22. Calculate total phosphorus concentration in the sample from following formula :

mg Phosphorus / g sample = Mean K x A x 0.781/1000

Figure 1C. Extract the food phytate with 2.4% HCl cover flask and shake at room temperature for 3 hrs.

Figure 2C. Prepare the column use glass barrell columns (0.7x15cm) equipped with a valve.

Figure 3C. Add 0.5 ml concentrated sulfuric acid, and 6.0 ml concentrated nitric acid to Kjeldahl flask.

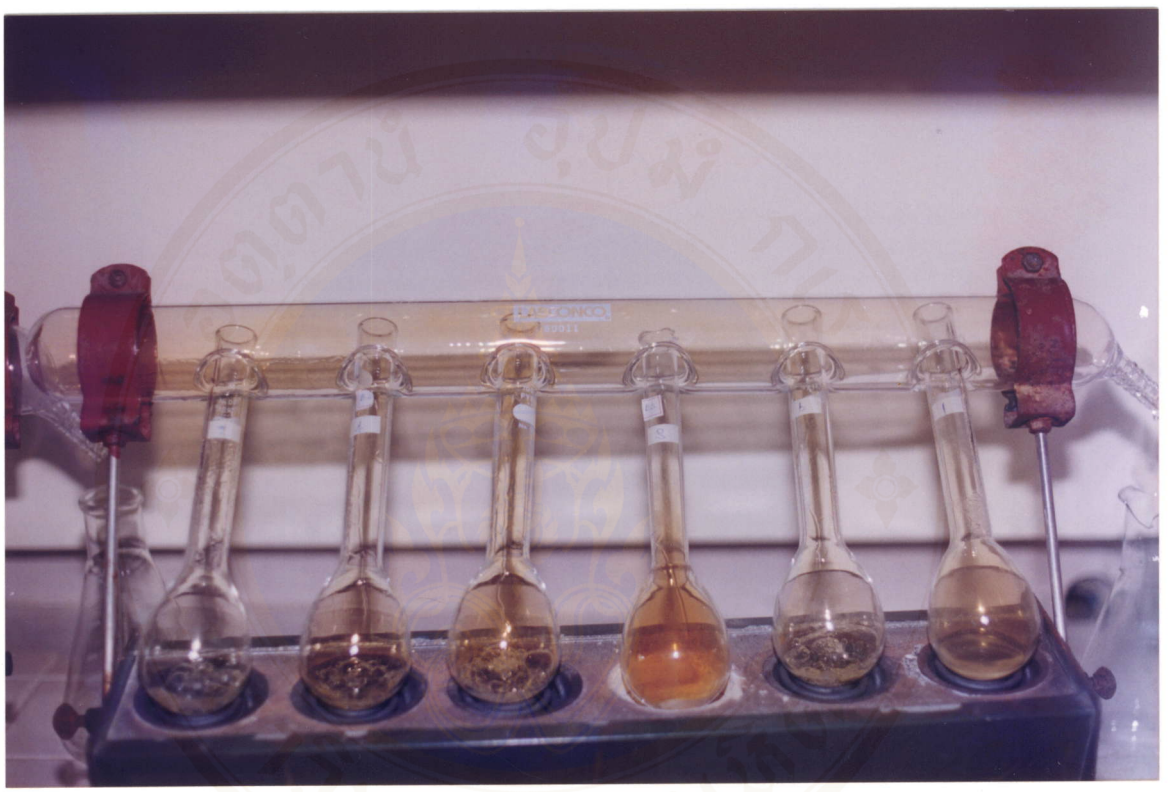

Figure 4C. Digest under hood on micro – Kjeldahl rack over medium heat.

Figure 5C. After digested the solution become dark brown colour.

Figure 6C. The blue colour was developed according to amount of phosphate after added molybdate-sulfonic acid solution.

Figure 7C. The optical density was read in spectrophotometer at 640 nm.

APPENDIX D

Determination of iron-binding phenolic compound

Principle

Phenolic compounds are extracted from food samples by dimethylformamide (DMF) in an acetate buffer. A ferric ammonium sulfate reagent is added and the resulting colour is read spectrophotometrically at two wavelengths corresponding to the absorbance maxima of Fe-catechol and Fe-galloyl complexes. Food blanks and reagent blanks are substracted.

Procedure

1. Weigh 0.5 – 2.0 g food sample into a 125 ml Erlenmeyer flask.
2. Add 50 ml 50% DMF-acetate solution.
3. The flask is carefully stoppened and shaken in a shaking machine for 16 hours at room temperature.
4. Remove sample from shaker and filter through a paper filter (Whatman No.541).
5. The food blank is prepared by mixing 2 ml of the filtrate with 8 ml of food blank reagent in a 15 ml test tube. Read against a blank consisting of 2 ml DMF-acetate and 8 ml of food blank reagent at both wavelengths.

6. 2 ml of the filtrate is vigorously shaken with 8 ml FAS – reagent in a 15 ml test tube.After 15 min the sample is read at 578 and 680 nm against reagent blank consisting of 2 ml DMF – acetate and 8 ml FAS – reagent.
7. Values for food blank are substracted from polyphenol extinction at each wavelength. The resulting net extinction values at 578 and 680 nm are used in the calculation.
8. Standard solutions containing tannic acid (TA) and catechin (C) are read with unknown samples, reagent blank and food blanks in each series.

Calculations

1. Abbreviations

1.1 Unknown samples :

N^{578} = Net sample extinction at 578 nm.

N^{680} = Net sample extinction at 680 nm.

SW = Sample weight in milligram.

1.2 Standard solution :

St. ext. TA 578 = Standard extinction tannic acid at 578 nm.

St. ext. TA 680 = Standard extinction tannic acid at 680 nm.

St. ext. C 578 = Standard extinction catechin at 578 nm.

St. ext. C 680 = Standard extinction catechin at 680 nm.

St. conc. TA = Standard concentration tannic acid µg/ml.

St. conc C = Standard concentration catechin µg/ml.

2. Calculations

2.1 Extinction ratios of standards

k_1 = St. ext. C 578 / St. ext. C 680

k_2 = St. ext. TA 680 / St. ext. TA 578

2.2 Calcution of content of tannins and catechins in unknown samples.

Step 1. Calculation of true extinctions

T ext. 578 = True extinction at 578 nm.

= Tannin extinction

The part of the extinction read at 578 nm originating from tannin in the food sample. The extinction at this wavelength orginating from catechin has thus been substracted.

T ext. 680 = True extinction at 680 nm.

= Catechin extinction

The part of the extinction read at 680 nm originating from catechins. The extinction originating from tannins at this wavelength thus been substracted.

$$\text{T ext. } 578 = \frac{N^{578} - k_1 N^{680}}{1 - k_1 k_2}$$

$$\text{T ext. } 680 = \frac{N^{680} - k_2 N^{578}}{1 - k_1 k_2}$$

Step 2. Calculation of amounts of catechin equivalents (mg/g sample)

and tannic acid equivalents (mg/g sample)

$$\text{Catechin equivalents} = \frac{\text{St. conc. C x 50 x T ext. 680}}{\text{SW x ST. ext. C 680}}$$

(mg/g sample)

$$\text{Tannic acid equivalents} = \frac{\text{St. conc. TA x 50 x T ext. 578}}{\text{SW x St. ext. TA 578}}$$

(mg/g sample)

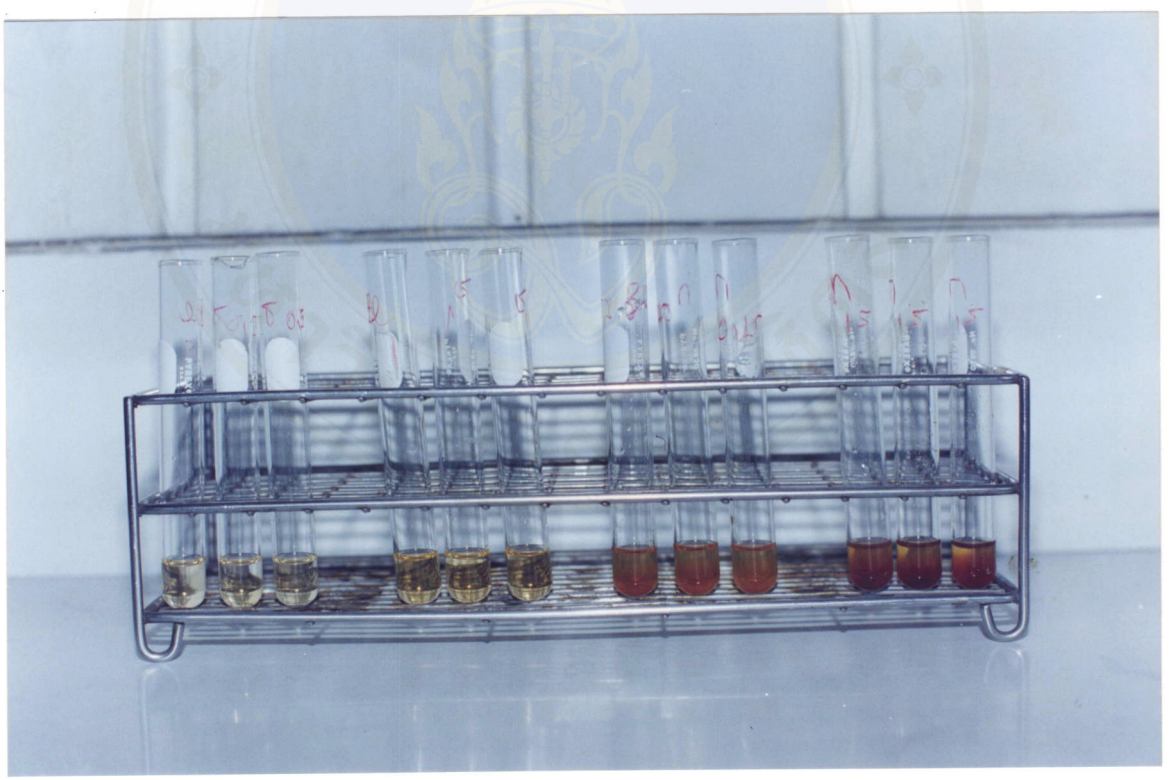

Figure 1D. Prepare tea and coffee in different dilutions.

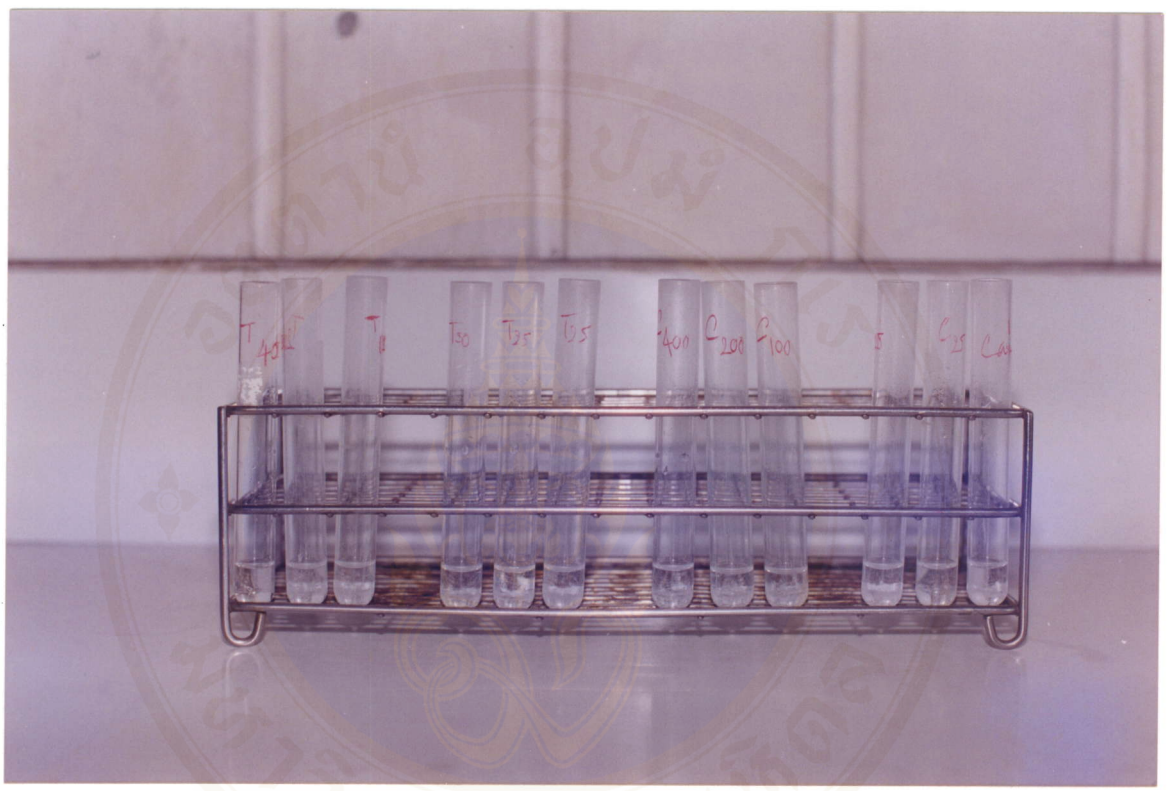

Figure 2D. Standard solutions containing tannic acid (TA) and catechin (C).

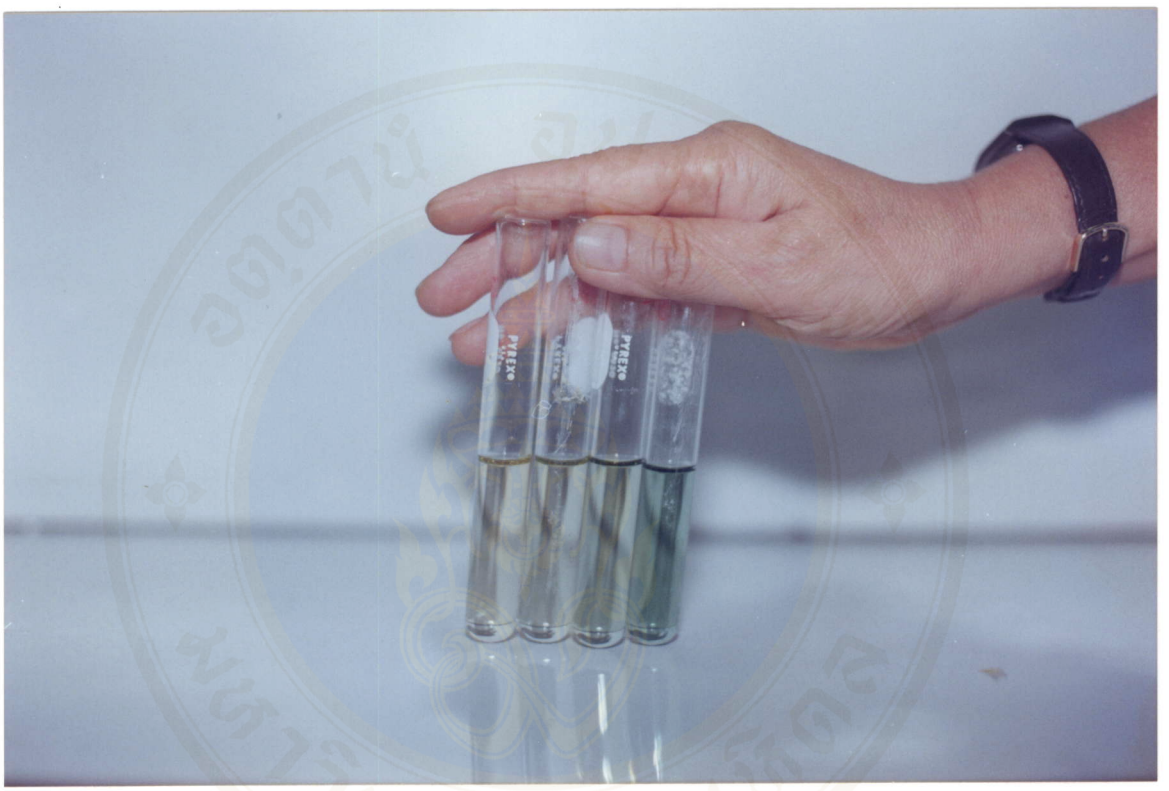

Figure 3D. The green colour was developed according to the amount of catechin.

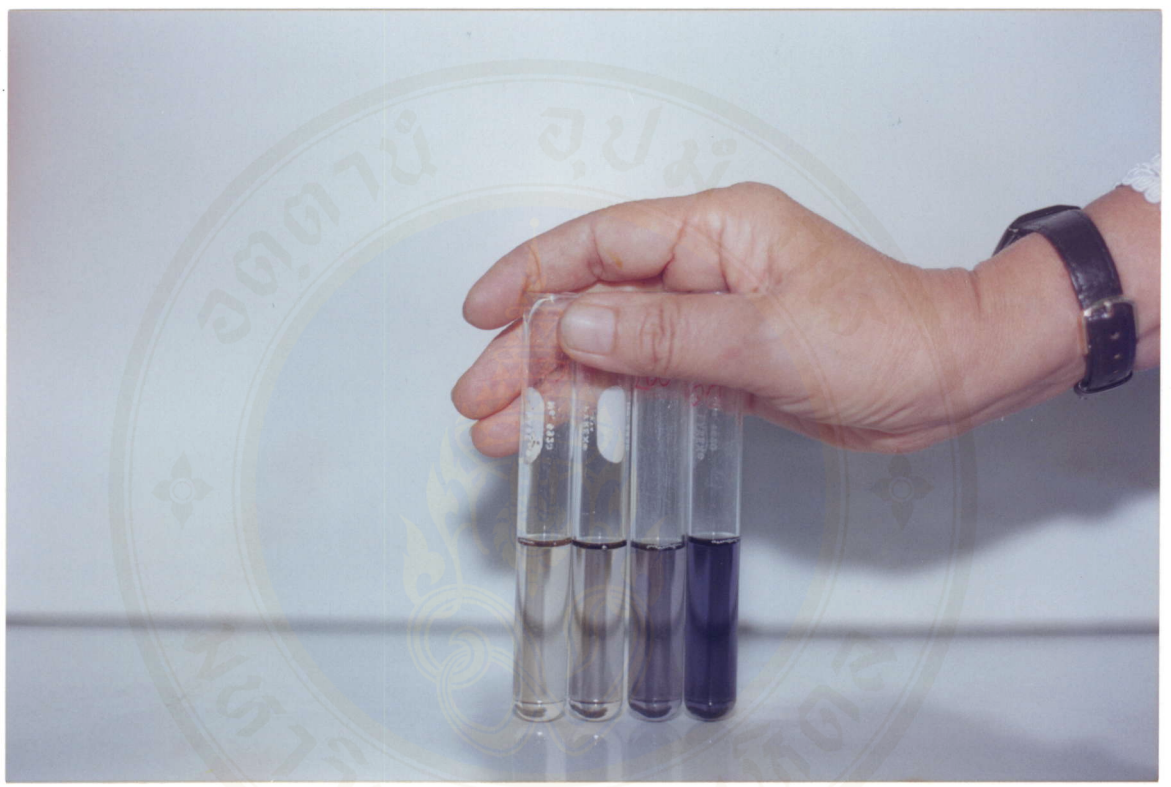

Figure 4D. The blue colour was developed according to the amount of tannin.

APPENDIX E

Preparation, name and components in the test meals

Meal preparation

The meal was prepared for one serving. All food items were carefully weighed using a direct reading Mettler balance. Boiled rice and simple steamed rice menus were prepared in the kitchen of the Nuclear Medicine Research Laboratory at Siriraj Hospital. The more complex steamed rice meals were supplied from hospital's kitchen.

Drinks preparation

Orange juice

Weigh 2 g of Tang orange add 30 ml of cold water, aliquot 25 ml into sample.

Coffee

Weigh 1.5 g of Nescafe Red Cup and 2 g of Mitrphol sugar add 30 ml of hot water, aliquot 25 ml into sample.

Tea

Weigh 3 g of Three Horses Bran Special Tea add 150 ml of hot water, leave for 20 minutes, aliquot 25 ml into sample.

Milk

Nong Pho UHT Fresh milk (unsweetened), aliquot 25 ml into sample.

Vitamax

Weigh 6 g of Vitamax add 30 ml of hot water, aliquot 25 ml into sample.

Vitamax – chocolate

Weigh 6 g of Vitamax – chocolate add 30 ml of hot water, aliquot 25 ml into sample.

Monie Gold

Weigh 6 g of Monie Gold add 30 ml of hot water, aliquot 25 ml into sample.

Monie Gold – chocolate

Weigh 6 g of Monie Gold – chocolate add 30 ml of hot water, aliquot 25 ml into sample.

Milo

Weigh 3 g of Nestle Milo add 30 ml of hot water, aliquot 25 ml into sample.

Ovaltine

Weigh 3 g of Ovaltine add 30 ml of hot water, aliquot 25 ml into sample.

Drinks preparation for the determination of iron – binding phenolic groups

Coffee

Weigh 0.5 g of Nescafe Red Cup add 10 ml of hot water, aliquot 0.1 ml for the determination.

Tea

Weigh 3 g of Three Horses Bran Special Tea add 150 ml of hot water, leave for 20 minutes, aliquot 0.1 ml for the determination.

Milo

Weigh 1 g of Nestle Milo add 10 ml of hot water, aliquot 0.1 ml for the determination.

Ovaltine

Weigh 1 g of Ovaltine add 10 ml of hot water, aliquot 0.1 ml for the determination.

Chocolate

Weigh 0.334 g of Van Houten Cocoa add 10 ml of hot water, aliquot 0.1 ml for the determination.

TABLE 21. Identification and description of the components, inhibitor and / or promotor in the test meals.

ID	Name	Major ingredients	Minor ingredients	Inhibitor and / or promotor
1	Boiled rice , Salted mustard green	Green mustard pickles		
2	Boiled rice , Pork dried	Pork dried		Pork
3	Boiled rice , Salted egg	Salted egg		Egg
4	Boiled rice , Fried egg	Fried egg	Sauce	Egg
5	Boiled rice , pork	Pork	Soup , vegetable dressing	Pork
6	Boiled rice , fish	Fish	Soup , vegetable dressing	Fish
7	Boiled rice , shrimp	Shrimp	Soup , vegetable dressing	Shrimp
8	Joke pork	Pork	Soup , vegetable dressing	Pork
9	Joke pork with egg	Pork , egg	Soup , vegetable dressing	Pork , egg
10	Rice , Pork soup	Pork , soybean curd	Soup , vegetable dressing	Pork , soybean curd
11	Rice , Kaitun	Egg	Soup , vegetable dressing	Egg
12	Rice , Fried fish	Fish	Sauce	Fish
13	Rice , Kaolao	Pork , pork liver , pork blood	Soup , vegetable dressing	Pork , pork liver , pork blood
14	Rice , Fried pork	Pork with garlic and pepper	Sauce	Pork
15	Rice , Fried pork liver	Pork liver with garlic and pepper	Sauce	Pork liver
16	Rice , Fried sponge gourd	Chicken , sponge gourd , egg	Sauce , fish sauce , oyster sauce	Chicken , egg
17	Rice , Fried meat	Meat , chinese kele	Sauce , oyter sauce	Meat

TABLE 21. (Continue) Identification and description of the components, inhibitor and / or promotor in test meals.

ID	Name	Major ingredients	Minor ingredients	Inhibitor and / or promotor
18	Rice , Fried shrimp with young corn	Shrimp , young corn	Sauce , oyster sauce	Shrimp , young corn
19	Rice , Chicken Pa - Lo	Chicken , egg	Soup (Pa – Lo)	Chicken , egg
20	Rice , Pork soup	Pork , soybean curd	Soup , vegetable dressing	Pork , soybean curd
21	Rice , Chicken curry	Chicken , chicken blood , egg plant , coconut milk	Chili paste	Chicken , chicken blood , egg plant
22	Rice , Pork soup	Pork , ashgourd	Soup , vegetable dressing	Pork
23	Rice , Celery cabbage soup	Pork , celery cabbage	Soup , vegetable dressing	Pork
24	Rice , Fried mungbean noodle	Pork , mungbean noodle	Tomato , Jew's ear mushroom	Pork , mungbean noodle
25	Rice , Chicken curry	Chicken , cabbage , coconut milk	Chili	Chicken
26	Fried rice , Fried chicken , ashgourd soup	Chicken , ashgourd	Lemon juice , tomato , cucumber , soup	Chicken
27	Rice , Pork green curry	Pork , pork blood , egg plant coconut milk	Chili paste	Pork , pork blood egg plant
28	Fried rice with small shrimp salted fermented celery soup	Small shrimp salted fermented , celery ,pork	Soup , vegetable dressing , egg , lemon juice , cucumber , shallot	Pork , small shrimp salted fermented
29	Rice , Fish (Tom–yum)	Fish (Platu)	Tomato , lemon juice , Chilli , vegetable dressing	Fish (Platu)

TABLE 21. (Continue) Identification and description of the components, inhibitor and / or promotor in test meals.

ID	Name	Major ingredients	Minor ingredients	Inhibitor and / or promotor
30	Rice , Pineapple curry	Pineapple , shrimp , coconut milk	Chili paste	Shrimp pineapple
31	Rice , Chicken green curry	Chicken , chicken blood , egg plant , coconut milk	Chili paste	Chicken , chicken blood ,egg plant
32	Rice , Kaitun , bitter melon soup	Egg , pork , bitter melon	Soup , vegetable dressing	Egg , pork
33	Rice , Kai Ka - Proa	Chicken	Chili , Holy Basil , sauce	Chicken
34	Fried rice , Fried chicken , cabbage soup	Chicken , crab , cabbage	Tomato , cucumber , soup	Chicken , crab

BIOGRAPHY

NAME Miss. Rujira Chokchai

DATE OF BIRTH 27 April 1974

PLACE OF BIRTH Yasothon, Thailand

INSTITUTIONS ATTENDED Mahidol University, 1992-1994:
Diploma of Science
(Radiological Technology)
Rajabhat Nakhonratchasima
Institution, 1995-1997
Bachelor of Science
(Public Health Science)
Mahidol University, 1998-2000
Master of Science
(Radiological Science)

POSITION & OFFICE 1994 –Present, Department of Radiology
Maharat Nakhonratchasima
Hospital and Medical Center
Nakhonratchasima, Thailand.
Position : Technician