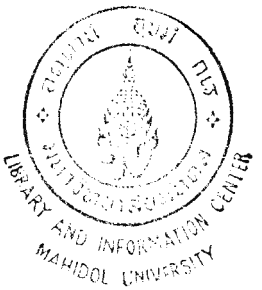

HEME AND NONHEME IRON CONTENT IN RAW AND COOKED ANIMAL PRODUCTS

PAWEENA NA PATTHALUNG

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

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Dietary iron is present in foods in two forms as heme and nonheme iron. Dietary heme iron is considered to be nutritionally important as it is more easily absorbed than nonheme iron. Animal sources have been considered to be an excellent source of dietary heme iron due to its high content. At present, information on the Thai Food Composition Table contains only total iron value with no separated heme and nonheme iron form. This information is necessary for estimating or predicting total available iron in a meal.

The objective of this study was to determine the concentration of total iron, heme and nonheme iron and vitamin C in animal products as affected by conventional cooking methods (boiling and steaming). All food samples were freshly purchased at three different shops in the same market from five local markets and two supermarkets in Bangkok during January to December, 1999. Heme and nonheme iron were analyzed by the Hornsey and Rhee methods, respectively. Total iron value was calculated by the summation of heme and nonheme iron. Vitamin C content was determined by AOAC method.

The results showed that there were large variations of nonheme iron content in pasteurization and ultra heat-treated milk ranging from 0.08– 0.18 mg/100ml whereas heme iron content was not detected. Heme iron was also not detected in both duck and hen's eggs while nonheme iron content was found in their egg yolks, ranging from 3.48 – 6.05 mg/100g wet weight. Cooked chicken breast and drumsticks were found to contain small amounts of heme iron (0.12 and 0.31mg/100g wet weight, respectively) and nonheme iron (0.27 and 0.63 mg/100g wet weight, respectively). Heme and nonheme iron in cooked beef loin were found to be 1.07 and 1.31 mg/100g wet weight. Liver is a good source of iron especially pork liver with approximately 20 – 30 % in form of heme iron. Cooked chicken and porcine blood curds were the best source of heme iron and the average was 9.17 and 15.38 mg/100 g wet weight, respectively with approximately 80 % of heme iron. Meatballs and sausage products were found to contain only small amounts of total iron, heme and nonheme iron. The richest sources of total iron were found in shellfish especially Sea mussels and Ark shells (14.73 and 17.73 mg/100g wet weight, respectively) with approximately 30 – 40 % of heme iron. Vitamin C in cooked samples was not detected except in cooked pork liver and chicken liver (2.24 and 3.00 mg /100 g wet weight). The effect of cooking in this study showed that heme iron level in the samples except for cooked blood curds was significantly reduced more than 50% by the boiling and steaming methods. Long heating exposure and small pieces of food also resulted in high losses of heme iron. In conclusion, this study indicated that cooked blood curds are the best animal source of dietary heme iron.

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ปวีณา ณ พัทลุง: ปริมาณธาตุเหล็กที่อยู่ในรูปของฮีมและไม่อยู่ในรูปฮีมในผลิตภัณฑ์จากสัตว์ที่ไม่ผ่านและผ่านกระบวนการทำให้สุก (Heme and nonheme iron content in raw and cooked animal products) คณะกรรมการควบคุมวิทยานิพนธ์ :รัชนี คงคาฉุยฉาย, Ph.D. สมศรี เจริญเกียรติกุล, D.Sc., นิภา โรจน์รุ่งวศินกุล, M.Sc. 173 หน้า ISBN 974-664-795-4

ธาตุเหล็กในอาหารมี 2 รูปแบบคือ เหล็กในรูปของฮีมและเหล็กที่ไม่ได้อยู่ในรูปของฮีม เหล็กที่อยู่ในรูปของฮีมจะถูกดูดซึมได้ดีกว่าเหล็กที่ไม่ได้อยู่ในรูปของฮีม เนื่องจากการดูดซึมไม่ขึ้นกับองค์ประกอบของอาหาร อาหารจากสัตว์เป็นแหล่งที่ดีของธาตุเหล็ก เนื่องจากมีธาตุเหล็กในรูปของฮีมในปริมาณสูง แต่ข้อมูลจากตารางแสดงคุณค่าอาหารไทยในปัจจุบันมีเฉพาะปริมาณของธาตุเหล็กเท่านั้นไม่ได้มีการจำแนกเหล็กในรูปของฮีมและเหล็กที่ไม่ได้อยู่ในรูปของฮีม ข้อมูลเหล่านี้มีความสำคัญต่อการประเมินการนำธาตุเหล็กในอาหารไปใช้ประโยชน์ได้ของร่างกาย

วัตถุประสงค์ของการศึกษาครั้งนี้คือวิเคราะห์หาปริมาณของธาตุเหล็กที่อยู่ในรูปของฮีม และเหล็กที่ไม่ได้อยู่ในรูปของฮีม และวิตามินซีในอาหารประเภทนม เนื้อสัตว์และผลิตภัณฑ์จากเนื้อสัตว์ที่ผ่านขบวนการทำให้สุกโดยวิธีการต้มและนึ่ง โดยสุ่มตัวอย่างอาหารจากร้านค้า 3 ร้านในตลาดเดียวกัน จำนวน 5 ตลาดและซุปเปอร์มาเก็ต 2 แห่ง ในกรุงเทพฯ ระหว่างเดือนมกราคมถึงเดือนธันวาคม 2542 และวิเคราะห์หาปริมาณธาตุเหล็กในรูปของฮีมและเหล็กที่ไม่ได้อยู่ในรูปของฮีมด้วยวิธีของ Hornsey และ Rhee ตามลำดับ ส่วนปริมาณธาตุเหล็กได้จากผลรวมของปริมาณเหล็กในรูปของฮีมและเหล็กที่ไม่ได้อยู่ในรูปของฮีม วิเคราะห์หาปริมาณวิตามินซีโดยวิธีของ AOAC

ผลการศึกษาพบว่า นมพาสเจอร์ไรส์และนมยูเอชที มีปริมาณธาตุเหล็กที่ไม่ได้อยู่ในรูปของฮีมระหว่าง 0.08 ถึง 0.18 มิลลิกรัมต่อ 100 มิลลิลิตร แต่ไม่พบปริมาณธาตุเหล็กในรูปของฮีม ในไข่แดง 100 กรัมน้ำหนักสดพบปริมาณธาตุเหล็กที่ไม่ได้อยู่ในรูปของฮีมระหว่าง 3.48 ถึง 6.05 มิลลิกรัม ไม่พบธาตุเหล็กที่อยู่ในรูปของฮีมในไข่ไก่และไข่เป็ด อกไก่และน่องไก่สุกพบธาตุเหล็กที่อยู่ในรูปของฮีมในปริมาณน้อยคือ 0.12 และ 0.31 มิลลิกรัมต่อน้ำหนักสด 100 กรัม และปริมาณธาตุเหล็กที่ไม่ได้อยู่ในรูปของฮีม 0.27 และ 0.63 มิลลิกรัมต่อน้ำหนักสด 100 กรัม ในเนื้อวัวสันนอกสุกพบปริมาณธาตุเหล็กที่อยู่ในรูปของฮีมและเหล็กที่ไม่ได้อยู่ในรูปของฮีม 1.07 และ 1.31 มิลลิกรัมต่อน้ำหนักสด 100 กรัม ตับเป็นแหล่งที่ดีของธาตุเหล็ก โดยเฉพาะตับหมูมีปริมาณธาตุเหล็กที่อยู่ในรูปของฮีมประมาณร้อยละ 20 ถึง 30 เลือดไก่และเลือดหมูต้มเป็นแหล่งที่ดีที่สุดของธาตุเหล็กที่อยู่ในรูปของฮีม มีค่าเฉลี่ยของธาตุเหล็ก 9.17 และ 15.38 มิลลิกรัมต่อน้ำหนักสด 100 กรัม โดยประมาณร้อยละ 80 เป็นธาตุเหล็กที่อยู่ในรูปของฮีม ในลูกชิ้นและไส้กรอกพบว่าธาตุเหล็ก เหล็กในรูปของฮีม และเหล็กที่ไม่ได้อยู่ในรูปของฮีมมีปริมาณเล็กน้อย ปริมาณของธาตุเหล็กสูงที่สุดพบในหอยโดยเฉพาะหอยแมลงภู่และหอยแครง คือมีปริมาณธาตุเหล็ก 14.73 และ 17.73 มิลลิกรัมต่อน้ำหนักสด 100 กรัมและคิดเป็นธาตุเหล็กในรูปของฮีมประมาณร้อยละ 30-40 ในการศึกษาครั้งนี้ ไม่พบวิตามินซีในตัวอย่างอาหารที่ปรุงสุก ยกเว้นในตับหมูและตับไก่สุก ซึ่งมีปริมาณวิตามินซี 2.24 และ 3.00 มิลลิกรัมต่อน้ำหนักสด 100 กรัม การประกอบอาหารโดยการต้มและการนึ่ง มีผลทำให้ปริมาณธาตุเหล็กที่อยู่ในรูปของฮีมในตัวอย่างอาหารลดลงมากกว่าร้อยละ 50 ยกเว้นในเลือดต้ม นอกจากนี้การประกอบอาหารที่ใช้เวลานาน และขนาดของอาหารที่มีขนาดเล็กมีผลทำให้ธาตุเหล็กที่อยู่ในรูปของฮีมมีปริมาณลดลงมาก ผลจากการศึกษาครั้งนี้อาจกล่าวได้ว่าเลือดต้มเป็นแหล่งที่ดีที่สุดของธาตุเหล็กในรูปของฮีม

CONTENTS

CONTENTS (cont.)

CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

Iron deficiency is one of the world's most common disorders and occurs when the amount of iron available is insufficient to meet an individual's needs. The estimation from the literatures review indicates that over 2.5 billion people in developing countries suffer from iron deficiency and more than half of them is anemic. The prevalence of anemia is over 50% among pregnant women, infants and children under the age of two years in developing countries. There have been a numerous guidelines and articles indicating that iron deficiency anemia is harmful to brain development and health throughout the life cycle. For example, growth retardation, lower resistance to infection and cognitive impairment are noted for infants and young children. In adults, anemia causes fatigue and diminishes work productivity. In pregnant women, it is associated with low- birth weight infants and increase prenatal mortality. In addition, iron deficiency inhibits the body's ability to regulate temperature when exposed to cold weather and it alters hormonal production and metabolism (1-7). The rational of iron deficiency has long been understood to result from the interaction of multiple etiological factors that lead to an imbalance between the iron requirements of the body and the amount of iron absorbed. The most important cause of iron deficiency is low bioavailability of dietary iron, particularly with high content of inhibitors and low intake of enhancers or good source of iron in diet. Accumulating evidence of iron deficiency anemia in developing countries demonstrate that the amount of iron potentially available from foods depends not only upon the amount of iron supplied but also the nature forms of iron and the composition of the diet with

which it is consumed (8). Therefore, only total iron content in the diet is not sufficient to indicate the adequacy of individuals with regards to iron. Accurate knowledge of the dietary iron intake of individuals or groups of people requires more information on the nutrient content of foods such as forms of iron (heme and nonheme iron), the amounts of inhibitors (phytate, milk, egg and polyphenols; tannin) and enhancers (vitamin C). Dietary iron presents in foods in two forms as heme and nonheme iron. Heme iron is considered to be nutritionally important as it is easily absorbed. The components of the diet do not affect its absorption. In contrast, dietary nonheme iron is poorly absorbed and some dietary factors interfere with its absorption. Monsen et al., suggested that the absorption of heme iron is 15-35%, depending on the iron storage, whereas only 2-8% is absorbed from dietary nonheme iron (9-11).

As mention above, there are the great differences in bioavailability and absorption between heme and nonheme iron. The relative quantities of heme iron and nonheme iron are needed to accurately estimate iron bioavailability, iron absorption or the amounts of total dietary iron in food. Therefore, the amount of heme and nonheme iron ingested must be considered separately. The composition of the diet has no influence on the amount of heme iron absorption, but cooking methods and cooking time have the effect on oxidizing the available heme into the less available nonheme iron (12-14). Various studies also showed that heating processing, storage, chemical treatment and cooking methods affected on vitamin C content in foods. The loss of vitamin C is caused by heat destruction during cooking process and/or oxidation during food preparation (15-16). Therefore, it is necessary to consider the basis of actual heme and nonheme iron and vitamin C concentration in raw, and cooked meats with their products normally consumed, i.e., fish, pork, beef, poultry, chicken and pork

liver and processed meat products. However, there is a limited information regarding to the value of heme and nonheme iron of these foods in Thai Food Composition Table.

The objectives in this study:

General objective:

To determine the effect of boiling and steaming on total iron, heme and nonheme iron and vitamin C content of animal products.

Specific objectives:

1. To determine the total iron, heme and nonheme iron and vitamin C content of animal products (before and after boiling and steaming).
2. To determine the effect of cooking methods (boiling and steaming) on heme and nonheme iron and vitamin C concentrations in animal products.
3. To compare the analyzed total iron content in each food item between two methods, the direct chemical analysis by AAS and the summation of heme and nonheme iron.

CHAPTER II

LITERATURE REVIEW

2.1 Iron

Iron is constituent of hemoglobin, myoglobin and a number of enzyme (17). It is the only nutrient that adult women have a greater RDA than adult men (18). A large proportion of body iron is hemoglobin, the color pigment of red blood cells that carries oxygen to various tissues, and it is associated with energy metabolism (19). The second large pool of iron is in the storage form, ferritin, which stored in the liver and reticuloendothelial cell, and erythroid precursors of the bone marrow. The storage levels vary according to an iron status. The stored iron may be mobilized to maintain functional iron compounds (20).

2.1.1 Function and Distribution

Iron in the body can be divided into functional iron, and nonfunctional iron. Over two third of body iron is usually in the form of functional iron. Most of functional iron is bound within the structure of hemoglobin, and small portion is bound within the structure of myoglobin.

Hemoglobin is the principal component of red blood cells, a metalloprotein with heme, an iron porphyrin, and attached to the protein moiety. Its function as an oxygen carrier from the lungs to the tissues and transport of carbon dioxide away from the cells to the lungs in the process of cellular respiration. It has a molecular weight of 64,500. The iron atom group must be in the reduced ferrous form to bind oxygen. Myoglobin

is a monomeric protein with a molecular weight of 16,900. It is an iron-protein complex, which is used for the short-term storage of oxygen.

The adult man and woman contains 35 to 50 mg of iron per kilogram body weight. The newborn infant contains relatively high level of iron about 70 mg/kg (21), which reflect the high levels of iron stored in ferritin and the relatively high concentration of red blood cells in the bloodstream of the neonate.

Hemoglobin contains about 60 percent of the body iron. Myoglobin contains about 4 percent of the body iron. The rest of functional iron is part of various enzymes that preformed varied acts of catalysis in cells. The cytochromes, present in all cells, do not combine with oxygen, but function in the respiratory chain in the transfer of electrons and storage through alternate oxidation and reduction of iron ($Fe^{2+} \rightleftharpoons Fe^{3+}$). Most of the nonfunctional iron in the body is held in iron storage compounds in the liver, spleen and bone marrow. Small portion of nonfunctional iron can be found as transport iron transferrin and iron within moleclues of ferritin (22).

Transferrin is a protein with a molecular weight of 74,000. It is an iron transport protein; distributed throughout most of extracellular fluid of the body with a continuous circulation from plasma to intestinal fluid and then back to the blood via the lymphatic vessels. The major functions of transferrin are to maintain extracellular iron in a soluble form that is suitable for cellular uptake and to regulate the supply of iron to cells. The amount of transferrin-bound iron in the plasma of a normal human is about 3 mg. The iron occurring in the storage protein ferritin represents between 5 and 30 percent of the body iron. Hemosiderin is an additional iron storage protein. This protein occurs in lysosomes and is thought to represent a partially degraded form of

ferritin. The distribution of iron-containing compounds in the human body is given in Table 1 (23).

Table 1 Distribution of iron containing compounds in the normal human adults.

Compound	Total in body (gm)	Iron content (gm)	Percent of total iron in body
Iron porphyrins			
(heme compounds)			
Hemoglobin	900	3.0	60-70
Myoglobin	40	0.13	3-5
Heme enzymes			
Cytochromes 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	0.4-0.8	15.0
Hemosiderin			
Total available iron stores		1.2-1.5	
Total iron		4.0-5.0	

Source: H.A. Harper, V.W. Rodwell, and P.A. Mayes (1979), (23)

2.1.2 Iron Metabolism

Iron is essential for the production of hemoglobin which function is to deliver oxygen from lung to tissues. It is also used for the synthesis of iron containing enzymes, which are required for the production of cellular energy (24). Body has three unique mechanisms for maintaining iron balance and preventing the development of iron deficiency: (1) the continuous reutilization of iron from cells catabolized in the body, (2) the presence of a specific storage protein, ferritin, which makes it possible to store iron in the body to meet excessive iron demands as in late pregnancy; and (3) the regulation of the absorption of iron affected by actual requirements with an increased iron absorption in the presence of iron deficiency and a decreased iron absorption in state of iron overload (25).

2.1.3 Iron Loss

The iron content of the body is normally constant by a delicate balance between intestinal iron absorption and iron losses. Basal iron losses mainly due to desquamation of surface cells from the skin, gastrointestinal and urinary tracts and the small amounts of gastrointestinal blood loss. Adult men loss iron about 0.9 g/day and 0.8 mg/day for women (11). In addition, during the childbearing years, iron is regularly loss through menstruation. Hallberg et al. (1992) showed that median monthly menstrual blood loss in the adult female is between 20 and 30 ml. (26). There are no data for infants and children, but iron losses may be proportionately greater than in adults because of the higher surface areas of the gastrointestinal tract and skin relative to body weight (11).

2.1.4 Iron storage and Transport protein

The major iron storage compounds are ferritin and hemosiderin, which are located primarily in the liver, reticuloendothelial cells, and erythroid precursors of the bone marrow. The synthesis of metabolical active compounds when body mobilization to satisfy increased body requirements is increased. The total amount of storage iron varies widely without apparent impairment of body function. Unless the stores are exhausted, the amount has no discernible influence on any physiologic or biochemical function other than iron absorption. Storage iron may be almost entirely depleted before iron deficiency anemia begins to develop.

The major role of transferrin is in the transport iron from the reticuloendothelial system and the intestine to the bone marrow for the synthesis of hemoglobin in developing red blood cells. A high proportion of diferric transferrin in the circulation and a rapid rate of erythropoiesis both favors increased iron delivery to the cell (27-28)

2.1.5 Iron Utilization

Hemoglobin of the red cell in the circulation has a finite life span approximately 120 days survival. The iron from the breakdown of hemoglobin can be almost completely re-utilized. Iron in the reticuloendothelial cell is predominantly from the catabolism of heme. Thus, the major flow of iron in the body is undirectional, passing in order, from transferrin to the erythroid marrow, to red blood cells and to reticuloendothelial cells. When iron is released from the reticuloendothhelial cells into the plasma, the iron is again bound to transferrin and the majority of it is returned to the erythroid marrow for heme synthesis. Approximately 85 percent of iron derived from the catabolism of erythrocytes are promptly returned to the plasma. Thus, there is only

a limited exchange with the storage iron in the reticuloendothelial cell under normal physiological circumstance (17).

2.1.6 Iron Absorption and Its Regulation

Because of the limited capacity of excretion iron of the body, iron homeostasis is maintained primary by adjusting iron absorption to body needs. Iron balance is mainly controlled at the site of intestinal absorption. The mechanisms by which the body increases the efficiency of iron absorption during the periods of iron need and decreases this absorption during times of iron overload are not completely understood. The absorption of iron is 0.5 to 1.5 mg/day mainly in the duodenum and jejunum even though the iron is normally ingested 10 – 20 mg/day. There are three factors for determination the amounts of iron absorption from the diet:

(a) the behavior of the intestinal mucosa,

(b) the body's need for iron

(c) the amount and nature of iron ingested and proportions of various other components (29).

2.2. Dietary Sources of Iron

Foods of animal origin are superior sources of trace elements because they are more available for absorption than from plant sources. Of all the seven essential trace minerals, iron is one of the most significant in human nutrition. Iron presents in foods in two forms as heme and nonheme iron. Dietary heme iron is considered to be nutritionally important as it is more easily absorbed than nonheme iron (30). Forty percent of all iron in animal tissues is considered to be heme iron, the remaining 60

percent of iron in animal tissues and all iron from vegetables sources is considered to be nonheme iron (Table 2).(31).

2.2.1 Heme Iron in the Diet

Foods derived from animal tissues are the major sources of heme iron. Hemoglobin and myoglobin are the main precursor proteins which heme iron is derived. Heme irons, a ferroprotoporphyrin, form an important source of dietary iron intake because of its high bioavailability. Heme iron in hemoglobin and myoglobin in meat represent 5 to 10 percent of dietary iron. In industrial countries, heme iron constitutes approximately 10 to 15 percent of ingested iron, and because of its high bioavailability, may account for about one-third of iron absorbed (32). Both heme and nonheme iron are markedly influenced by iron states. Rates of absorption are inversely related to the quantity of iron stores. Monsen et al. (1978) suggested that the absorption of heme iron is 15-35% depending on the iron store whereas only 2-8% form of dietary iron nonheme iron (9).

2.2.2 Nonheme Iron in the Diet

Nonheme iron compounds as found in foods of both plant and animal origins. Iron is present in plants in three main forms as follows:

(i) metalloproteins with the predominant example being plant ferritin (33).

(ii) soluble iron in the sap of xylem, phloem and vacuoles

(iii) a non-functional form complexed either to structural components or storage compounds predominantly in the form of phytates (31).

Nonheme iron in animal-derived food is found in many forms including ferritin and hemosiderin in meat product, bound to the phosphoprotein, phosphovitin in egg yolk

and in milk bound to lactoprotein (31). Nonheme iron in cereals, vegetables, fruits, roots, pulses and beans forms the main part of dietary iron. The absorption is influenced by the individual iron status and the composition of the diet (34-35).

Table 2 Dietary sources of iron (mg).

Meats (40% heme iron, 60% nonheme iron)			
Bacon, 3 strips (84g)	0.6	Kidney,3 slides (98g)	9.5
Beef hamburger (112g)	3.3	Lamb chop (98g)	3.0
Beef, porterhouse (224g)	3.8	Liver, calf (70g)	9.0
Brains (84g)	2.0	Pork chop (98g)	4.4
Frankfurter(49g)	0.6	Spareribs (84g)	2.3
Ham, fresh (98g)	2.3	Sweetbread (98g)	1.2
Heart (98g)	5.5	Veal cutlet (98g)	4.2
Fish and shellfish (40% heme iron, 60% nonheme iron)			
Clams (98g)	3.4	Sardines (98g)	5.2
Crab (98g)	0.8	Scallops (98g)	3.0
Crayfish (98 g)	1.5	Shrimp (98g)	2.0
Flounder (98g)	1.4	Snail, raw (98g)	3.5
Haddock(98g)	1.2	Tuna (98g)	1.9
Salmon(98g)	1.2		
Poultry and game (40% heme iron, 60% nonheme iron)			
Chicken(98g)	1.5	Quail (98g)	3.8
Duck (98g)	5.8	Squab (98g)	1.8
Pheasant(98g)	3.7	Turkey (98g)	3.8

Table 2 Dietary sources of iron (mg) (cont.).

Legumes and vegetables (nonheme iron)			
Beans, red kidney (98g)	2.4	Chickpeas (98g)	6.9
Beans, green lima (98g)	2.8	Dandelion greens (98g)	3.1
Beans, green snap (98g)	0.8	Fennel (98g)	2.7
Broccoli (98g)	0.8	Kale (98g)	2.2
Cauliflower (98g)	1.1	Lentils (98g)	2.1
Cabbage (98g)	0.4	Spinach (98g)	3.1
Dried fruits and nuts (nonheme iron)			
Apricots (98g)	5.5	Almonds (98g)	4.4
Oats (98g)	3.0	Cashews (98g)	3.8
Figs (98g)	3.0	Peanuts (98g)	3.0
Prunes (98g)	3.9	Walnuts, black (98g)	6.0
Raisins (98g)	3.5	Walnuts, English (98g)	2.1
Cereals and cereal products (nonheme iron)			
Barley (2tbsp) ~ 28 g	0.5	Oatmeal (1cup) ~240 g	1.7
Bulgar (2tbsp) ~ 28 g	1.0	Noodles (1cup) ~160 g	1.4
Farina (1 cup) ~ 245 g	2.0	Wheat germ (3 tbsp.) ~18 g	2.6

Source: From C. F. Church and H.N. Church (1975), (36)

2.3. Absorption of Dietary Iron

Iron absorption is an issue of continuing interest in the nutritional sciences because of the relatively high frequency of iron-deficiency anemia and the remarkably poor efficiency of absorption of most forms of dietary iron. The amounts of iron absorbed are determined by the amounts of heme and nonheme iron in the diet and the balance between different factors enhancing and inhibiting the absorption as well as the behavior of the intestinal mucosa. The mucosal cells necessarily take up not all the available iron within the lumen of the intestine. Their behavior is related to the iron content of the body, so that the percentage absorption rises as the body iron content falls.

Variations in the bioavailability of food iron are of greater importance for iron than the amount of iron in the diet. The two forms of food iron have different mechanism for absorption (37).

2.3.1 Heme Iron Absorption

The structure of heme iron was shown in Figure 1. The absorption of heme iron is constant and independent of meal consumption. Heme iron is absorbed by a different mechanism from the nonheme iron. It is absorbed into intestinal epithelial cells as a metalloporphyrin ring, which may be facilitated by a vesicular transport system (24,39). Many investigators showed that heme is taken up by mucosal cells after it has been released from its globin combination by proteolytic duodenal enzymes. Globin degradation products are important in maintaining heme in a soluble state so it is available for absorption. After heme iron enter the intestinal absorptive cells, it is

rapidly degraded by heme oxygenase, and the iron released then enters to the common intracellular iron pool as inorganic iron (40-42) (Figure 2).

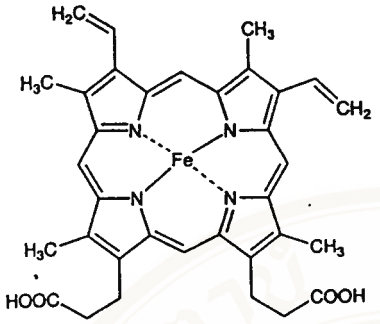


Figure 1 Structure of heme iron.

Source: The Merck Index 1996 (38).

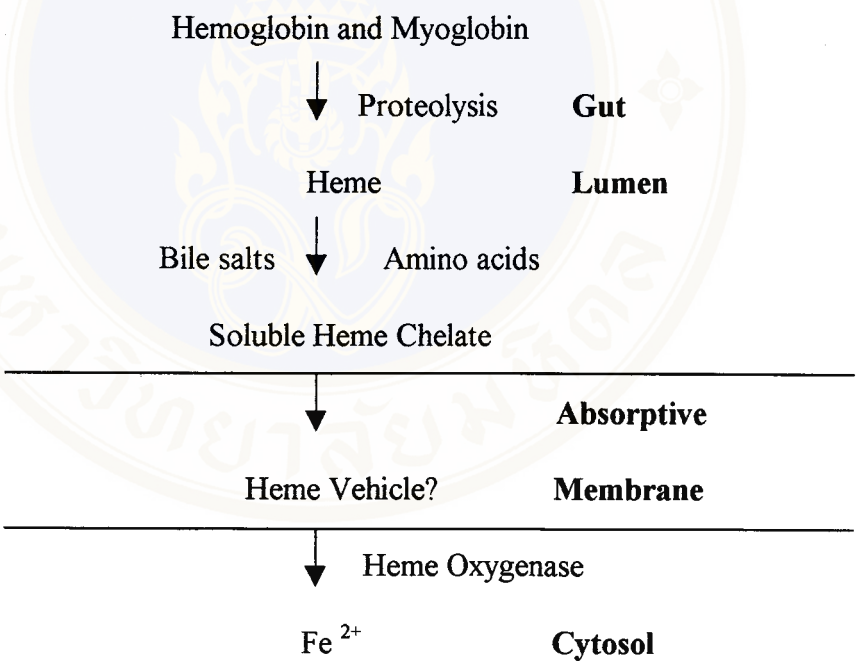


Figure 2 Absorption of heme iron.

Source: Uzel C et al. 1998 (39).

The efficiency of heme absorption is not influenced by the other dietary factors. Hallberg and colleagues showed that calcium inhibited the delivery of ingested heme iron to the blood by inhibiting the transport of the iron through absorptive membrane, rather than by inhibiting the actual process of absorption into the cells (26).

2.3.2 Nonheme iron absorption

Nonheme iron is associated with a variety of dietary compounds. Mechanism for absorption of nonheme iron starts in the stomach. The hydrochloric acid content of gastric juice is important for solubilizing of nonheme iron; the absorption is reduced when acid secretion decreased (43). During peptic digestion, nonheme iron complexes were splitted and iron is free into a pool where it may be reduced rapidly form chelates or rendered insoluble. Following neutralization in the duodenum, the more soluble iron in the pool will be better available for absorption. The dissolved ion irons then bind to transferrin- like iron binding proteins, which are then carried into the cells of the gastrointestinal wall by specific transporters. All dietary forms of nonheme iron ingested in the same meal from a common pool within the intestinal lumen (18). Absorption from this pool is determined not by the type of the iron ingested but by enhancers and inhibitors present in foods. Enhancers promote absorption by maintaining iron in a soluble form. Inhibitors bind iron or make iron insoluble and prevent its uptake by brush border of the intestinal (44).

Table 3 Dietary factors that affect iron absorption.

Enhancers	**Inhibitors**
Vitamin C	Phytate
Acid in stomach	Lack of stomach acid
Heme iron	Oxalate
High body demand for red blood cells	Tannins (in tea)
(blood loss, high altitude, physical	Excess of other minerals (Cu, Zn, Mn,
training, pregnancy)	Ca)
Low body iron stores	Full body iron stores
Meat protein factors	Some antacid drug
	Phosvitin in egg yolk

Source: Wardlaw MG et al. 1990 (45)

The percentage of nonheme iron that is absorbed from a meal can vary from less than 1 to more than 50 %. The consuming heme iron and nonheme iron together increased nonheme iron absorption (45). Percentage absorption of heme iron is 5-10 fold higher than that of nonheme iron, although heme represents only 10-15 % of dietary iron (42).

2.3.3 Factors Affecting Nonheme Iron Absorption

Many different dietary factors can either enhance or inhibit the absorption of the nonheme iron within food (Table 3); the major factors are as follows.

(a) Enhancing Factors

Ascorbic Acid:

The potent dietary factor enhancing iron absorption is ascorbic acid. Its promoting effect is strong dose-related either in the form of natural or synthetic ascorbic acid (46-47). The mechanisms related to its effect on iron absorption are:

(i) Reducing effect on ferric iron to more soluble ferrous state.

(ii) Ability to form iron-ascorbate chelates which preserve the iron solubility in the more alkaline duodenal pH (48).

The enhancing effect of ascorbic acid on nonheme iron absorption has been tested in a number of dietary settings. It has been shown to play a critical role in diets in which little or no meat is present and its effect seems to be more pronounced in meal containing much inhibitors (49). Moreover, timing of consumption both nonheme iron and ascorbic acid is equally important. If nonheme iron absorption is to be increased via this factor, must be consumed together.

The effect of ascorbic acid seems to be independent effect when compare with other promoters of iron absorption, such as meat. Cooking, baking and prolong warming of food can completely or partially destroyed the ascorbic acid (16,50).

Citric, malic, tartaric, lactic, and other organic acids also enhanced nonheme iron absorption (50).

Meat, Poultry, Fish and Seafood:

Meat in the diet not only contributes highly available heme iron but also increases the absorption of other food iron. The enhancing effect of meat and fish was

first reported by Layrisse et al. in 1968 (51). Several investigators have also studied to clarify the mechanism of the enhancing effect by meat. The researchers suggest that meat may enhance iron absorption in two ways. The first, iron from hemoglobin and myoglobin is more readily available because globin digestion products formed during the digestive process prevent nonheme iron polymerization and maintaining iron in a more soluble form. The second, meat enhances iron absorption by increasing the transfer of iron through the mucosal cells because meat contains with enhancing factors such as cysteine, cysteine containing peptides, dipeptides, other peptide and digestion products (52-60). Of special interest is the observation by Mulvihill et al. in 1998 showed that the " meat factor " may be a-SH- rich proteolytic digestion product which are also enhanced the iron absorption (60). Meat or degradation products are also related to some indirect mechanism of iron absorption due to an effect on the secretion of gastric or intestinal juices or the rate of gastric emptying (54).

(b) Inhibiting Factors

A number of factors have been identified which limited nonheme iron absorption from the common pool. These include phenolic compounds, phytates, the dietary fiber complex, phosvitin, calcium and phosphorus (61-63).

Phenolic Compounds:

Phenolic compounds are relative substances with its active site being the hydroxyl groups. In most cases, phenolic compounds can be found in a characteristic feature of all plant tissues (64). However, there is wider diversity of phenolic compounds in plant tissues. As for the plant polyphenols, which are heterogeneous groups of natural products, the polyhydroxyphenolic compounds designated as

vegetable tannins are a distinctive group of plant polyphenols. They are structurally divided in to 2 groups as follows:

(i) A number of derivatives of flavanols that generally are the largest group of phenolic compounds, so-called nonhydrolysable or condensed tannins.

(ii) Esters of a sugar, hydrolysable tannins which mostly known in the form of glucose.

As the matter of fact, while all vegetable tannins are phenolic compounds, but not all-phenolic compounds are vegetable tannins (65). There are different patterns of hydroxylation in phenolic compounds. The iron binding properties of phenonlic compounds have a very special interesting pattern. Molecules with aromatic rings bearing two hydroxyls (catechol group) or three hydroxyls (galloyl group) positioned at adjacent carbon atoms have iron binding properties. The formation of iron phenolic complexes in the intestine is presumably the basis of the interaction of phenolic compounds with iron absorption. The condensed tannin of which catechin is an example, occurs widely in various fruits and vegetables. It has one catechol and one-resorcinol groups on the phenolic molecules. There are one more galloyl groups' tannins in their molecules of hydrolysable. The study of Brune et al. (1989) showed that gallic acid and tannic acid (containing galloyl groups) strongly inhibited iron absorption in man, while catechin had no effect (66).

Tannins, which are present in tea, coffee, certain spices, sorghum and certain vegetables, are powerful inhibitors of nonheme iron absorption (67). The study of Disler et al. (1975) showed that when Indian tea was fed with a variety of meals, it was shown to inhibit iron absorption in man (62). The *in vitro* and *in vivo* work

provide evidences that the interference of iron absorption by phenolic compounds is probably due to Fe- phenolic complex formation in the gastro-intestinal lumen making the iron less available for absorption (68). Many investigators suggested that phenolic compounds inhibit iron absorption in a dose-related manner (61,69). However, ascorbic acid was able to overcome this inhibitory effect. The study of Siegenberg et al. (1991) on iron absorption from a white- bread meal with various concentrations of tannin showed that as little as 12 mg tannic acid decreased iron absorption by one-third and 50 mg reduced absorption by almost 70 percent (69). The maximal suppression of about 80 percent obtained with much larger doses. It has been shown in the same study that more than 50 mg ascorbic acid would be required to overcome the inhibitory effect on iron absorption of any meal containing more than 100 mg tannic acid. Nearly the same time, a typical Southeast Asian meal was studied by Tuntawiroon et al. (1991) to examine the effect of a very popular vegetable in Thailand, Yod Kratin, (*Leucaena glauca)* which contains a considerable amount of iron binding phenolic groups (70). The results showed that there was a 50 percent decrease in iron absorption after the administration of as little as 3 g Yod Kratin, corresponding to 87.6 mg tannic acid equivalents. Adding of ascorbic acid partly counteracted inhibition, 100 mg ascorbic acid reduced inhibition of iron absorption from 5 g Yod Kratin by half and the inhibition from 10 g Yod Kratin by quarter.

Phytate:

Phytate is a compound, which naturally occurs in many foods derived from plants. It is major phosphorus compound in plant seeds and/or grains and is also found in significant quantities in roots and tubes. Phytate exists in plant mostly as

inositol phosphate, the most abundant form is phytic acid (71). In addition several studies indicated that there is strong dose-effect relationship between inhibition of iron absorption and the content of phytate (66).

In 1989, Brune et al. evaluated that the effect of sodium phytate on the absorption of iron by adding varies doses of sodium phytate in to a meal. The results showed that there was a 50 – 70% decrease in iron absorption after consuming 10 and 50 mg sodium phytate. They also found a reduction in iron absorption by 85% after adding 250 mg sodium phytate to a meal by this level, vitamin C cannot counteracts this effect (66).

Gillooly and Co-workers (1983) also demonstrated that phytate is indeed a major inhibitors in cereal foods (61). It has been postulated that phytate interfere with iron absorption by reacting with the iron as it passes through the intestinal tract, and that phytate forms highly insoluble complexes with iron.

Calcium:

Calcium is metallic element, taken into the body as a constituent of certain foods, especially dairy products that are the most efficient source of calcium. Concerning of iron absorption, numerous studies have shown that calcium, even in small amount, had an inhibitory on iron absorption (72-74).

According to the study of Cook et al. (1991) the effect of various calcium salts commonly used as supplements on the absorption of dietary nonheme iron and iron supplements was determined. When taken without food, calcium carbonate did not inhibit the iron absorption of ferrous sulfate at either 300 mg Ca and 37 mg Fe

or 600 mg Ca and 18 mg Fe. However, calcium citrate and calcium phosphate at the latter dose reduced absorption by 49% and 62% respectively (75).

Hallberg and colleagues (1992) studied on the dose-effect relationship between calcium content and iron absorption in 126 human subjects. These authors demonstrated that dose of calcium chloride (between 40 mg and 600 mg calcium) caused a dose related reduction (up to 300 mg calcium) in nonheme iron absorption from a meal of wheat rolls containing 10 mg native calcium and 3.8 mg iron by 50-60%. This influence was strongly dose-related but no further inhibit with higher amounts. They also found a reduction in heme iron absorption by 50-60% after addition of 165 mg Ca as milk cheese or calcium chloride to a hamburger meal (26).

Egg :

Eggs are almost the cheapest and easiest to be found for most part of the world. They also are nutritionally an important source of dietary protein. Nevertheless, iron in egg is poorly available to human. The more important concern is about the egg yolk Phosphoprotein as a name phosphovitin containing in the egg yolk inhibits absorption of iron (76). Cook and Monsen in the same study (1976) have found that when meat is replaced with egg and egg albumin; there is a marked reduction in nonheme iron absorption (8). The experiment showed that, when the quantity of egg albumin was doubled, the absorption of iron in semisynthetic meal dropped. When it is completely omitted from the formulation the absorption increased about threefold (77). Bjorn-Rasmussen et al. (1972) reported that iron absorption from omelet is about 1.46 percent (78). The related study reported by Miller et al. (1981)

also supported that in a semisynthetic meal containing egg white or egg albumin had significantly higher bioavailability on iron absorption than the whole egg meal (79).

The main difference in composition between egg albumin and the other sources of animal protein is the lack of nucleoproteins in egg albumin and the composition of compounds formed during digestion. During the digestion of egg albumin iron-bioavailability-inhibiting-substances are released which masks the enhancing influence of animal proteins. It appears that these "inhibiting-substances " may be related to the release of partially or non- digested high molecular weight peptides, which tightly bind the Fe (II) formed, thus inhibiting its transport across the membrane (60).

2.4. Requirement and Recommendation

Iron requirement varies widely in different individuals depending upon their age and sex, nutrition and state of health. Women of fertile age having the greatest need of iron. The iron need is estimated by measuring the loss from the body and determining the amount of dietary iron needed to replace the loss. The total excretion of iron is about 1 mg per day. An additional 0.5 mg is assumed to be needed by women each day to compensate for menstrual losses. Since the amount of iron absorbed is believed to be 10 percent, 10 mg daily is recommended for all adult men and for women 51 years of age and over; 15 mg is recommended for females 11 to 50 years of age (80). The higher allowance for women of child-baring age would permit the accumulation of iron to satisfy the demand of pregnancy which are greater than can be supplied with

ordinary diet, supplemental iron is desirable. The iron need for age and sex groups expressed as RDA in mg and Thai RDA are given in Table 4 and Table 5, respectively

Table 4 Recommended daily dietary allowances for iron.

Group	Age (years)	RDA (mg)
Infants	0.0-0.5	6
	0.5-1.0	10
Children	1-3	10
	4-6	10
	7-10	10
Males	11-14	12
	15-18	12
	19-22	10
	23-50	10
	51+	10
Females	11-14	15
	15-18	15
	19-22	15
	23-50	15
	51+	10
Pregnant		30
Lactating		
1st 6 month		15
2 nd 6 month		15

Source: Recommended Dietary Allowances 1989 (80).

Table 5 Recommended dietary allowances of iron (Thai RDA).

Group	Age (months)	Iron (mg)
Children	3-5	6
	6-8	7
	9-11	8
	(Year)	
Boy-Girl	1-9	10
Boy	10-15	12
Male	16	10
Female	10-49	15
	50	10
Pregnant		30
Lactating		15

Source: Thai RDA (1989), (81)

2.4.1 Thai Recommended Daily Intake – Thai (RDI)

Thai RDI recommended that iron intake in Thais at the age over 6 years old should be 15 mg per day (Thai RDI 1995) (82).

2.5. Effect of Cooking on Iron Content

Numerous studies (14,83-86) showed that heat decreased heme iron content and increased nonheme iron content in meat or meat extracts. Schricker and Miller (1983) suggested that heating accelerate oxidative cleavage of the porphyrin ring thereby

allowing released of the iron from heme complex (87). Time (59,83,88), final temperature (88-90), rate of heating (12), method of cooking (59,87,89), surface area of food sources (87,91) and O_2 during cooking (92) influenced the released of heme iron from meat pigments.

Buchowski et al (1988) and King et al (1990) indicated that slow and longer time heating increased the amount of nonheme iron than fast heating and shorter time (12,83). The same results in the studies of Hayakawa et al (1983, 1986) suggested that the longer time of hemoglobin exposure under high temperature, the more porphyrin was destroyed and the more nonheme iron content was increased (93,94).

The reduction of heme iron content caused by cooking may vary from 10 to 100% depending on the cooking time, type of cooking and meat (Table 6)(14). Schricker and Miller (1983) and Jansuitivechakul et al (1985) also indicated that heme reduction was less than 10% during normal cooking (braising and roasting) and 40% during very high heating (baking or microwave) (87,89). Gutbrie and Dieciano (1995) suggested that the best ways to prevent iron loss from food iron by preparing food in large pieces rather than finely chopped and cooking food by steaming or microwave rather than boiling (95).

Table 6 Heme iron as percentage of cooking losses.

Meat	Cooking methods	%Heme iron loss
Beef,		
Ground	Pan fry	12
Round	Braise	47
Loin	Broil	15
Pork,		
Loin	Broil	47
Picnic	Roast	16
Lamb,		
Chop	Broil	12
Chicken,		
Breast	Broil	17
Thigh	Broil	28
Turkey,		
Ground	Pan fry	30
Rabbit	Pan fry	62

Source: Carpenter et al. 1995 (14).

2.6. Vitamin C

L-ascorbic acid (or vitamin C) is a white crystal simple compound having formula as $C_6H_8O_6$. It's molecular weight is 176.13 and is very soluble in water but less soluble in ethyl alcohol and most lipid solvents. L-ascorbic acid or free ascorbic acid (AA) is known to be the unstable among all vitamins as it can be reversibly oxidized in the body to more stable L-dehydroascorbic acid (DHA). However, the latter compound still fully possesses the vitamin C activity. Another isomer of L-ascorbic acid, named erythobic acid (D-isoascorbic acid) possesses little or no vitamin C activity but has a similar redox potential. Both DHA and erythobic acid are widely used as an antioxidant by air, light and heat, especially in an alkaine medium and in the present of Cu as a catalyst.

2.6.1 Absorption, Transport, Storage and Excretion

L-ascorbic acid is absorbed in the intestine by a sodium-dependent and active transport process. At low doses, ascorbic acid can be almost completely absorbed. However, only 80-90% will be absorbed when the amount of ascorbic acid intake is as approximately consumed in normal daily life 30 to 60 mg per day (96). The absorbed ascorbic acid is transported in the plasma and stored in the cells as a free anion. It freely enters and stores in the cells including leukocytes and the red blood cells. The total body store of vitamin C can vary from less than 20 mg to approximately 3,000 mg depending on the amount of L-ascorbate consumed. When 60 mg of vitamin C is consumed per day (the recommended dietary allowance for adults), the total body store is around 1,500 mg (97). The body stores is normally several folds higher than those found in blood plasma (Table7)(48).

Urine is the major excretory route of ascorbic acid in man. It is excreted in the form of both ascorbate and its metabolites. These identified metabolites are oxalate, dehydroascorbate, ascorbic acid-2-sulfate, diketogulonic acid, methyl ascorbate and 2-ketoascorbitol.

Table 7 Ascorbic acid levels of adult human tissues.

Tissue	Ascorbic acid (mg/100 g wet tissue)
Adrenal glands	30-40
Pituitary gland	40-50
Liver	10-16
Spleen	10-15
Lungs	7
Kidneys	5-15
Testes	3
Thyroid	2
Heart muscle	5-15
Skeletal muscle	3-4
Brain	13-15
Pancreas	10-15
Eye lens	25-31
Plasma	0.4-1.0
Saliva	0.07-0.09

Source: Combs GF (1998), (48).

2.6.2 Requirement of Vitamin C

The recommended dietary allowances (RDA) as established by the U. S. National Research Council is 60 mg for both adult males and females as well as those who are pregnant and lactating women. The allowance for infant is based on the amount supplied daily by 850 ml of human milk or 35 mg of ascorbic acid. The allowance for children from 1 to 12 years of ages is 40-mg daily (98).

2.6.3 Sources of Vitamin C

A relatively high concentration of ascorbate is found in vegetables, fruits and in animal organs such as liver and kidney. Milk contains small amounts of vitamin C, thus cannot be counted on. Eggs, cereal grains, sugar, fats, nuts, dried legumes, and dried fruits contain either very little or none at all.

Table 8 Vitamin C content of some animal sources.

Food groups	Ascorbic acid (mg/100g)
Meats	0-2
Poultry	0-1
Fish	0-0.1
Egg	0
Liver	10-40
Kidney	10-40
Milk, whole, pasteurized	0.8-1
Milk, semi-skimmed, pasteurized	0.8-1
Milk, skimmed, pasteurized	0.8-1
Milk, whole, sterilized	trace

Source: Combs GF (1998), (48)

2.6.4 Effect of Cooking on Vitamin C

Heating is the most common method for preparing meat, but vitamin C can be destroyed by heating since vitamin C is easy oxidized especially on exposure to heat and leached into the cooking water or soaking water (99). Meat and meat product are also not good source of vitamin C (100-101). In addition the review literature of Apiradeewajeeset (1996) reported that the loss of vitamin C can be occurred from cooking 0-100% (100).

The shorter boiling time and the smaller amount of water used, the lesser vitamin C is lost during cooking (15). The losses of vitamin C contents also depend on the numerous other factors, including the nature of the food, its reaction in the cooking

environment (acid or alkaline medium), the period and degree of heating. Especially the extent to which food is exposed to water and air in the cooking process (102).

CHAPTER III

MATERIALS AND METHODS

3.1 Samples

Food items in this study were selected by the criteria following from the Fourth National Nutrition Survey of Thailand by the Ministry of Public Health in 1995 (103). These criteria are

(i) Amount and frequency of consumption

(ii) Popularity of food consumption and food availability

Table 9 Food commodities selected for analysis in this study

Food items	
Meat and Poultry	**Beef**: loin **Pork**: tenderloin, loin and liver **Chicken**: breast with skin, drumsticks and liver
Processed meat products	**Blood curd**: cooked porcine blood curd, cooked chicken blood curd **Meatballs**: cooked beef ball, cooked chicken ball, cooked pork ball and cooked fish ball **Sausages**: Smoked and non-smoked pork and chicken sausage

Table 9 Food commodities selected for analysis in this study (continued).

Food items	
Seafood and Fish	**Shellfish**: Ark shells, Mussels and Baby clam **Marine fish**: Red snapper, Fresh short-bodied mackerel, steamed short-bodied mackerel **Fresh water fish**: Fresh water catfish, Striped snake- head fish and Nile tilapia **Squid**: Splendid squid **Shrimp**: Giant tiger prawn
Milk	Pasteurized, ultra heat treated milk(UHT) Soybean milk
Egg	**Hen egg**: White, yolk and whole egg **Duck egg**: White, yolk and whole egg

3.1.1 Sample purchased

The samples were purchased in the morning from five local markets and two supermarkets in Bangkok (Table 10).

Table 10 Name of five markets and two supermarkets where the food samples were purchased.

Local markets	Supermarkets
Sam-yan	Central Plaza Supermarket
Salanamron	Tang-Hua-Seng Supermarket
Tae-ved	
Saphan-kwai	
Ramintra	

(a) Meats and Cooked Blood Curds:

Meats, cooked chicken and porcine blood curds were randomly purchased in the morning at the three shops in the same market from five local markets in Bangkok during June to August 1999. Each sample was purchased at least 0.5 kilogram and analyzed individually on the same day.

(b) Processed Meat Products:

Meatball: the meatballs (beef, chicken, fish and pork) were purchased and analyzed individually on the same day.

Sausage products: the sausage samples produced in Thailand, smoked and non-smoked pork and chicken sausages, were randomly purchased from two supermarkets in Bangkok (Table 10).

(c) Seafood, Marine and Fresh water fish:

Each type of seafood, marine and fresh water fish at least 0.5 kilogram was randomly purchased from five local markets during September to November 1999. Each was analyzed individually on the same day.

(d) Milk:

Five brands of different flavors of the pasteurized and ultra heat-treated milk (UHT) and two brands of soybean milk were purchased from two supermarkets. Each was analyzed individually on the same day.

(e) Eggs:

Duck and hen eggs were randomly purchased at least 8 eggs at the three different shops in the same market from five local markets during March to May 1999.

3.1.2 Sample preparation

Meats, cooked blood curds, seafood and fish were washed with tap water several times in order to remove the slime and blood. Samples were cut into pieces, only the edible parts of samples were selected and washed again with deionized distilled water and drained on the stainless sieve until dried. The food samples were then divided into two portions. One portion was analyzed as fresh sample and the other was boiled or steamed or blanching prior to analysis. Weights of the samples before and after cooking, cooking water and other residues were recorded. The edible portion of each sample was homogenized in electrical blender (Moulinex masterchef model 375). The homogenized samples were then immediately determined individual for moisture, vitamin C, heme and nonheme iron. For total iron analysis, each sample from the three different shops in the same market was pooled together to form one sample each.

General descriptions of the procedures used for the sample preparation were shown in Figure 3.

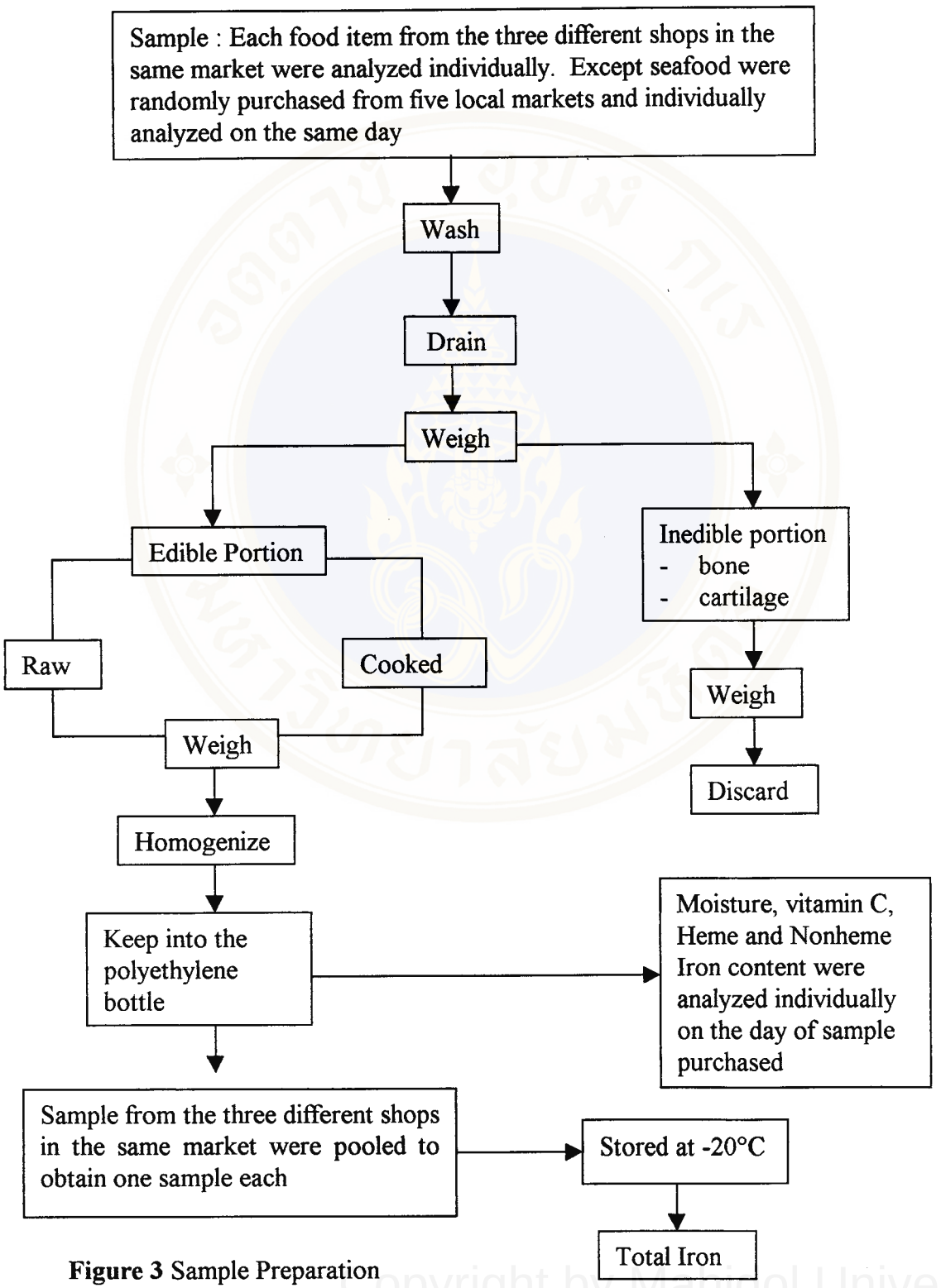


Figure 3 Sample Preparation

(a) Chicken and Chicken liver:

Breast and drumsticks: Each breast, drumsticks, and liver was washed with tap water several times and the last time with deionized distilled water and drained on the stainless sieve, and then weighed. The edible parts of each were randomly separated into two portions and reweighed. One portion was analyzed as raw sample and the other was boiled before analysis. Each edible portion of breast was weighed on average 36.6 grams each (Appendix 3).

(b) Beef, Pork and Pork liver:

Each edible part of beef, pork and pork liver was cut into small pieces and weighed approximately for 40-50 grams *per* each and divided into two portions (Appendix 3). One portion was analyzed as raw sample and the other portion was analyzed after boiling.

(c) Processed Meat Products:

Meatballs and sausages were cut and divided into two portions and weighed. Each portion was then washed with deionized distilled water and drained on the stainless sieve and reweighed.

(d) Seafood, Marine and Fresh water fish:

Seafood and fish were prepared as commonly household cooking techniques. Fresh water and marine fishes were placed on the chopping board to scrape off the scales. Then washed under running water, and removed gills, eviscerate and head. Cut the topside of fish and carefully removed pieces from the rib bones. The flesh of fish were then washed with deionized distilled water and drained on the

stainless sieve, weighed and cut into pieces. These pieces were randomly separated into two portions and reweighed.

Shellfish:

Ark shell (Hoi Khraeng), Mussel (Hoi Malang Phu) and Baby clam (Hoi Line) were selected as commonly consumed shellfish. The shellfish were soaked under tap water for a long time to remove mud, sand and dirt. Any broken-shell or open (dead) shellfish were discarded. The samples were then washed again with deionized distilled water and drained on the stainless sieve and weighed.

Giant tiger prawn:

Giant tiger prawns (Kung kuladam), were washed with tap water several times to remove the slime and ice. Each prawn was peeled and cut its back the lengthwise down to remove back vein. After washing with deionized distilled water, the prawns were drained on the stainless sieve and weighed. Finally, the prawns were randomly separated into two portions and reweighed. One portion was analyzed as raw sample and the other was boiled before analysis.

Splendid squid:

Splendid squid (Pla meuk klung) has a tubular body, tentacles and covered with a thin skin flecked with red. Their clear bone, skin, eyes and ink sac were removed. The tubular body, head and tentacles were washed with distilled water and deionized distilled water and drained on the stainless sieve and weighed. They were cut into two pieces and randomly divided into two portions and weighed again.

(e) Eggs:

Each kind of eggs was washed with tap water and distilled water to remove dirt. Four eggs were carefully flipped with spatula and separated into white and yolk parts. For the whole egg preparation, four eggs of each kind were flipped with spatula or spoon and homogenized with homogenized blender.

(f) Milk:

Five brands of different flavors of the pasteurized and ultra heat- treated milk (UHT) and two brands of soybean milk were individually determined for moisture, heme, nonheme iron and vitamin C contents.

3.1.3 Cooking Methods

Foods were prepared for edible portion. The choice of cooking method depends on the individual or cultural dietary habits. Thailand has a variety of cooking methods for meats, meat products, seafood, fish and egg i.e., boiling, frying, steaming, blanching, and broiling. The data from the cook book or cooking survey suggested that boiling, blanching, steaming and broiling are the popular ways of preparing animal and animal products or commonly used in household (100-101,104-105). Therefore, in this study, the boiling method was used to evaluate the effect of cooking on heme, nonheme iron and vitamin C contents in meat, meat products, prawn, squid, short-bodied mackerel and eggs. The steaming method was used for evaluation of fresh water and marine fish, Mussels and Baby clam and the blanching for Ark shells (Table 11).

(a) Boiling Method

Samples (meat, liver and meat products, squid, short-bodied mackerel, shrimp and eggs) were boiled by conventional method. The deionized distilled water approximately 800 ml was poured into aluminum containers and covered with the lid and then placed on the gas stove until water was boiled. The samples were put in the boiling water and covered tightly until the samples become tender (1 to 20 minutes depends on the nature of each sample). To test for tenderness, insert a fork into the thickest part of samples and twist gently. If this was done, the samples will flake very easily. The samples were placed on the dish and measured the internal temperature. The samples were allowed to cool before weighing. The boiling time and internal temperature of each sample were showed in Table 11.

(b) Steaming Method

Distilled water was added into the aluminum steamer pot and covered with the lid (water should not touch bottom of the basket) and placed on the gas stove and let stand until boiling. The samples (only the edible portion of fresh water and marine fish and mussels and carper shells) were placed on the steamer basket until the sample became tender. To test for tenderness by inserting a fork into the thickest part of the samples and twist gently. When done, the samples will flake very easily. The samples were removed and placed on the container and measured the internal temperature. The samples were allowed to cool and weighed. The doneness of mussels and baby clam, was showed when, the shellfish curled around the edges or until the shell was opened. The samples were then allowed to cool and weighed. The shell and fiber were loosen and discarded away. Only the edible portions of each were

selected and reweighed. The steaming time and internal temperature of each sample were showed in the Table 11.

(c) Blanching Method

Deionized distilled water approximately 800 ml were poured into aluminum containers and covered with the lid until water become boil. After that, ark shells were immersed in boiling water for 1 and 3 minutes. The samples were drained on the stainless sieve until cool and weighed. The shell was loosened and only the edible portions were selected to analyzed and weighed. The blanching time was showed in Table 11.

Table 11 The internal temperature and time used for the sample cooking.

Food items	Methods	Internal temperature (°C)	Time (minute)
Chicken:	**Boiling**		
Breast		75-85	5
Drumsticks		85-90	18-20
Liver		78-83	5-6
Pork:			
Loin		75-78	5-7
Tenderloin		76-83	5
Liver		77-85	8
Beef:			
Loin		80-86	10
Processed Meat products:			
Meatballs		70-72	3
Sausages		70-74	3
Cooked porcine blood curd		60-64	1
Cooked chicken blood curd		74-75	3
Eggs:			
Hard egg		72-75	5

Table 11 The internal temperature and time used for the sample cooking (continued).

Food items	Methods	Internal temperature (°C)	Time (minute)
Seafood and fish:			
Fresh short-bodied mackerel	**Steaming**	60-65	2.5
Giant tiger prawn		62-66	2.5
Splendid squid		74-79	3
Red snapper		60-63	15
Fresh water catfish		65-72	15
Strip snake head-fish		73-80	15
Nile tilapia		76-80	15
Mussels		61-65	10
Baby clam		52-55	7
Ark shell	**Blanching**		1
Ark shell			3

3.2 Analytical Methods

All food samples were individually determined for moisture, heme and total iron and vitamin C contents in duplicate analysis. For nonheme iron, it was individually determined in triplicate analysis.

3.2.1 Moisture Determination

Moisture content of sample was determined in hot air oven at 100 ± 5 ° C (106,107). The samples were dried under specific condition until constant weight is

obtained. The moisture content was calculated from the sample weight loss (Appendix 4).

3.2.2 Vitamin C Determination

Vitamin C was determined by fluorescence spectrometer (108). The sample was extracted with metaphosphoric acid. After extracting, the sample was transferred to a flask containing norit, pipetted the filtrate into flask containing Sodium acetate (NaOAc) and borate solution. Left to stand for 15 min, take 5 ml of each filtrate to flask containing NaOAc solution and dilute to volume with water. The fluorescence of samples and standard solution were measured by using fluorescence spectrometer, excitation wavelength 350 nm, emission wavelength 430 nm.(Appendix 5).

3.2.3 Heme Iron Determination

Heme iron was determined by using the method of Clark et al.(1997) and Hornsey method (1956) (109-110). The sample (2-5 g) was placed into a 50 ml of centrifuge tube, and 20 ml of acetone and 0.5 ml of Conc. HCl were added. Water was added so that total water in the tube, both from the sample and from the added water, equaled 4.5 g. The mixture of sample was homogenized by ice homogenizer at 100 × 100 *rpm* for 1 minute (Nussei Am- 1) and filtrate was measured by spectrophotometer at 640 nm. Water content in the sample was determined by drying at 100 ± 5° C (Appendix 6).

3.2.4 Nonheme Iron Determination

Determination of nonheme iron content in the sample was followed the method of Rhee K.S. et al. (1987) (111). The samples (2-5 g) were weighed into glass centrifuge sealed screw-cap tubes. The extraction solution (15 ml of a 1:1 mixture of 40 % TCA/

6N HCl) and 0.2 ml of 0.39 % $NaNO_2$ was added. The mixtures were mixed well with vertex and the tubes were sealed with screw cap and placed in shaking boiling water bath at 65° C for 18-20 hours. After cooling, the mixture solutions were centrifuged at 2500 *rpm*, 4 °C for 10 minutes and the supernatants were filtrated. The nonheme iron concentration of the filtrates were determined by spectrophotometer at 540 nm (Appendix 7).

3.2.5 Total Iron Determination

Total iron was determined by wet digestion method. The solution of samples was determined by using Atomic Absorption Spectrophotometer. (Appendix 8)

3.2.6 Calculation of Heme Iron Retention

True retention of heme iron in cooked food was calculated as described by Murphy et al. (1975) (112). True retention means percent of heme iron content that retains in food after cooking. The following formula was used:

$$\% \text{ True retention} = \frac{(\text{nutrient content per g of cooked meat} \times \text{g of meat after cooking}) \times 100}{(\text{nutrient content per g of raw meat} \times \text{g of raw meat})}$$

3.2.7 Statistical analysis

For descriptive statistics, mean, standard deviation, median and range were computed for heme and nonheme iron content. Mann-Whitney U test was used to identify significant cooking effect for each iron content. All computations performed by using SPSS for windows version 9.01 software. Results were considered significant when $p < 0.05$. (113).

3.3 Quality Assurance of Analytical Data

Percent recovery and in house control materials were used for the quality control of analytical data. Porcine blood, liver powder and soybean powder which prepared in our laboratory and hematin (# H 3505) which purchased from Sigma company, were used as in house control sample for heme and nonheme iron and total iron determination. Tang® Natural fresh orange, flavor instant drink was used as reference material for vitamin C. Therefore, the control charts of vitamin C, heme and nonheme iron and total iron were obtained by analysis in every series of the determination using the values of mean ± 2SD (Table 12). Values within 2SD were used for all nutrients and the percent recoveries for all nutrients analysis in this study were between 95 to 100%. Therefore, the results showed in Table 13 to 14 indicated that all the methods, which were selected, for nutrients analysis from this study can be used as the reference methods for determination of heme and nonheme iron, total iron and vitamin C. Triplicate analysis of analytical samples in this study was carried out with precision of less than 10 percent difference.

Table 12 Mean ± 2SD in mg/ 100g of in-house quality control samples.

Nutrients	**Control sample**	**Mean ± 2SD**
Total iron	Liver powder	59.13 ± 2.81
	Soy bean powder	7.73 ± 0.45
Heme iron	Hematin	10220 ± 237
	Porcine blood powder	124 ± 27
Nonheme iron	Liver powder	49.85 ± 3.11
Vitamin C	Tang® Natural fresh orange	1056.43 ± 110.57

The validity for analysis of nonheme iron in foods was evaluated by the method of Rhee et al. (1987) (111). Homogeneous fresh liver was used as model of sample studies. Ferric chloride standard, which is equal to 50 – 100% of nonheme iron content in a sample, was added before acid treatment. Percent recovery of nonheme iron was then examined. The recoveries of nonheme iron were found to range from 95 – 97 % (Table 13).

To test the validity of the method for determinations of heme iron, hematin (15 μg/g liver and hemoglobin (4 mg) were used as the internal standard. Percent recoveries of heme iron were showed in Table 14 ranging from 95 - 97%. Bovine liver (NIST 1577a) obtained from the Nation Bureau of Standard were used as reference material for total iron analysis. The analysis values of total iron in bovine liver were equivalent to certified standard (192 ± 5.1 μg/g vs 196 ± 20 μg/g).

Table 13 Recovery of nonheme iron in liver by following the method of Rhee et al., 1995.

Sample	Nonheme iron (μg/g wet wt.)	%
Liver	69.2 ± 1.2	
Liver + 30 μg Ferric chloride	97.0 ± 2.0	95 - 97

Values are reported as mean ± SD for replicates analysis.

Table 14 Recovery of heme iron in liver by following the method of Clark et al., 1997.

Sample	Heme iron (μg/g wet wt.)	(%)
Liver	37.7 ± 1.4	
Liver + hematin (150 μg/g liver)	50.6 ± 2.6	95
Liver + hemoglobin (4 mg)	51.1 ± 1.2	97

Values are mean ± SD for triplicate analysis.

Heme iron content in one gram of hemoglobin is 3.35 mg.

One microgram of heamatin is equal to 0.0882 μg of heme iron per gram of sample.

CHAPTER IV

RESULTS

4.1 Moisture Content Analysis

The moisture contents of raw and cooked foods from animal origin determined by thermal drying method are shown in Table 15 -22.

Table 15 shows the moisture contents of pasteurized, UHT and soybean milk. Moisture content of these milk were within the same magnitude, approximately 83-88%.

Table 16 shows the moisture contents of raw hen and duck eggs, which were slightly higher than those of cooked hen and duck eggs. Moisture contents of raw and cooked egg white for both hen and duck eggs were higher than egg yolk approximately 39%.

Table 17-18 shows similar moisture contents of raw meat and poultry, ranging between 72 to 76 percent. After cooking, the moisture contents were slightly lower than raw meat and poultry with approximate 12%.

Table 19-20 shows the moisture contents of raw and cooked meatballs and sausages that ranged from 60-81 % and 59-62%, respectively. The moisture contents before and after cooking seemed to be unchanged.

Table 21 shows the moisture contents of cooked porcine and chicken blood curds. Percent of moisture contents of cooked porcine and chicken blood curd as purchased

were similar (92%). The cooking times have no effect on percent moisture content of both blood curds.

Table 22 shows the moisture contents of raw and cooked seafood. The moisture contents of raw marine and fresh water fish ranged from 72-79%, while the cooked items contained the moisture between 65-73%. The moisture contents of raw and cooked shellfish, squid and prawn were higher than fish.

4.2 Vitamin C Content Analysis

Vitamin C contents of raw and cooked foods from animal origin are shown in Table 15-22.

There was no vitamin C in milk, eggs, meatballs, sausages, porcine and chicken blood curds (Table 15, 16, 19, 20and 21).

Table 17-18 shows the effect of cooking on vitamin C contents of raw and cooked meats and poultry. Vitamin C contents in raw meat and poultry were similar, approximately 0.08 mg/100g. Vitamin C contents were not found in cooked meat and poultry. Vitamin C contents in raw and cooked pork and chicken liver were ranged from 1.84-3.00 mg/ 100g wet weight. Vitamin C contents of boiled pork and chicken liver were slightly higher than that of raw pork and chicken liver, with approximate 16-20% probably due to a moisture loss.

Table 22 shows the effect of cooking on vitamin C contents of raw and cooked seafood. Raw muscle tissue of seafood contained vitamin C approximately 0.08 mg/100g at the same level as of animal muscles meat. Vitamin C contents were not found in cooked seafood, except in 1 minute boiled Ark shells.

Table 15 . Moisture and vitamin C content of milk.

Food Items	No. of sample	Moisture content (%)	Vitamin C (mg/100 ml)
Milk:			
Pasteurized milk	15	87.89 ± 0.47	ND
Pasteurized sweet milk	15	84.36 ± 0.79	ND
Pasteurized chocolate milk	15	83.24 ± 0.55	ND
Pasteurized strawberry milk	15	83.42 ± 1.03	ND
Ultra heat-treated milk	15	88.21± 0.79	ND
Ultra heat-treated sweet Milk	15	84.60 ± 0.37	ND
Ultra heat-treated chocolate milk	15	83.20 ± 0.70	ND
Ultra heat-treated soybean milk	6	84.74 ± 0.91	ND

The reported values are mean ± SD.

ND: not detected.

Table 16 Moisture and Vitamin C content in eggs.

Food Items	No. of sample	Cooking methods	Cooking time (min)	Moisture content (mg/100g wet weight)		Vitamin C content (mg/100g wet weight)	
				raw	cooked	raw	cooked
Hen egg							
Whole egg	15	Boiling	5	76.22 ± 0.75	74.65 ± 0.87	ND	ND
Yolk	15	Boiling	5	50.44 ± 0.82	48.87 ± 0.72	ND	ND
White	15	Boiling	5	87.57 ± 0.81	86.94 ± 0.80	ND	ND
Duck egg							
Whole egg	15	Boiling	5	69.90 ± 0.89	67.77 ± 1.40	ND	ND
Yolk	15	Boiling	5	46.78 ± 0.88	45.11 ± 0.87	ND	ND
White	15	boiling	5	86.79 ± 0.60	86.01 ± 0.61	ND	ND

The reported values are mean ± SD.

ND: not detected.

Table 17 Moisture and vitamin C content (per 100 g edible portion) of raw and cooked meats.

Food Items	No. of sample	Cooking method	Cooking time (min)	Moisture content (g)		Vitamin C content (mg)	
				raw	cooked	raw	cooked
Beef:							
Loin	13	Boiling	10	76.42 ± 2.33	61.11 ± 1.75	0.07 ± 0.10	ND
Pork:							
Tenderloin	15	Boiling	5	75.66 ± 1.34	68.87 ± 2.57	0.08 ± 0.08	ND
Loin	15	Boiling	5-7	73.71 ± 1.05	66.59 ± 1.54	0.09 ± 0.08	ND
Liver	15	Boiling	8	72.94 ± 1.21	68.73 ± 1.95	1.84 ± 0.63	2.24 ± 0.77

The reported values are mean ± SD.

ND: not detected.

Table 18 Moisture and vitamin C content (per 100 g edible portion) of raw and cooked poultry.

Food Items	No. of sample	Cooking method	Cooking time (min)	Moisture content (g)		Vitamin C content (mg)	
				raw	cooked	raw	cooked
Poultry:							
Breast	15	Boiling	5	76.47 ± 1.08	70.21± 0.99	0.07 ± 0.09	ND
Drumsticks	15	Boiling	18-20	75.53 ± 2.55	69.94 ± 1.19	0.08 ± 0.07	ND
Liver	15	Boiling	5-6	76.44 ± 0.98	72.50 ± 1.81	2.58 ± 1.16	3.00 ± 1.04

The reported values are mean ± SD.

ND: not detected.

Table 19 Moisture and vitamin C content (per 100 g edible portion) of raw and cooked meatballs.

Food Items	No. of sample	Cooking method	Cooking time (min)	Moisture content (g)		Vitamin C content (mg)	
				raw [a]	cooked	raw [a]	cooked
Processed meat products:							
Meatball:							
Cooked beef ball	5	Boiling	3	73.20 ± 3.19	72.85 ± 3.09	ND	ND
Cooked chicken ball	7	Boiling	3	61.37 ± 4.32	60.49 ± 4.74	ND	ND
Cooked pork ball	12	Boiling	3	71.92 ± 2.98	71.02 ± 3.08	ND	ND
Cooked fish ball	11	Boiling	3	81.73 ± 3.10	81.73 ± 3.11	ND	ND

The reported values are mean ± SD.

ND: not detected.

[a] already processed

Table 20 Moisture and vitamin C content (per 100 g edible portion) of raw and cooked sausages.

Food Items	No. of sample	Cooking method	Cooking time (min)	Moisture content (g)		Vitamin C content (mg)	
				raw [a]	cooked	raw [a]	cooked
Processed meat products: **Sausages:**							
Smoked chicken sausage	4	Boiling	3	60.97 ± 2.13	59.87 ± 1.99	-	-
Non-smoked chicken sausage	5	Boiling	3	62.66 ± 3.35	61.74 ± 3.09	-	-
Smoked pork sausage	6	Boiling	3	60.14 ± 1.90	59.32 ± 2.33	-	-
Non-smoked pork sausage	6	Boiling	3	61.36 ± 3.08	60.81± 3.04	-	-

The reported values are mean ± SD.

-: Not determined.

[a] already processed

Table 21 Moisture and Vitamin C content in cooked blood curds.

Food Items	No. of sample	Cooking method	Moisture content (mg/100g wet weight)			Vitamin C content (mg/100g wet weight)		
			raw[a]	boiled (1 min)	boiled (3min)	raw[a]	boiled (1 min)	boiled (3min)
Cooked blood curd:								
Cooked porcine blood curd	15	Boiling	92.80 ± 0.46	91.73 ± 0.51	90.46 ± 0.33	ND	ND	ND
Cooked chicken blood curd	15	Boiling	92.66 ± 1.34	92.11 ± 1.35	91.65 ± 1.41	ND	ND	ND

The reported values are mean ± SD.

ND: not detected.

[a] already processed

Table 22 Moisture and vitamin C content in raw and cooked of selected seafood (mg/100g edible portion).

Food Items	No. of samples	Cooking method	Cooking time (min)	Moisture content (mg/100g wet weight)		Vitamin C content (mg/100g wet weight)	
				Raw	cooked	raw	cooked
Shellfish:							
Ark shells	5	Boiling	1	-	83.64 ± 3.93	-	0.18 ± 0.18
	5	Boiling	3	-	87.65 ± 1.48	-	ND
Mussels	5	Steaming	10	87.65 ± 1.48	73.09 ± 2.09	-	ND
Baby clam	5	Steaming	7	-	74.27 ± 2.63	-	ND

The reported values are mean ± SD.

ND: not detected.

-: Not determined.

Table 22 Moisture and vitamin C content in raw and cooked of selected seafood (mg/100g edible portion) (continued).

Food Items	No. of samples	Cooking method	Cooking time (min)	Moisture content (mg/100g wet weight)		Vitamin C content (mg/100g wet weight)	
				Raw	cooked	raw	cooked
Marine fish:							
Red snapper	5	Steaming	15	79.14 ± 0.50	73.11 ± 0.68	ND	ND
Fresh short- bodied mackerel	5	Steaming	2.5	74.37 ± 4.42	71.34 ± 3.82	0.06 ± 0.07	ND
Steamed short-bodied mackerel	5	-	-	-	65.59 ± 3.70	ND	ND

The reported values are mean ± SD.

ND: not detected.

-: Not determined.

Table 22 Moisture and vitamin C content in raw and cooked of selected seafood (mg/100g edible portion) (continued).

Food Items	No. of sample	Cooking method	Cooking time (min)	Moisture content (mg/100g)		Vitamin C content (mg/100g)	
				raw	cooked	raw	cooked
Fresh water fish:							
Nile tilapia	5	Steaming	15	77.69 ± 1.15	73.12 ± 0.88	0.02 ± 0.02	ND
Fresh water catfish	5	Steaming	15	72.37 ± 1.75	69.59 ± 1.80	ND	ND
Striped snake-head fish	5	Steaming	15	76.25 ± 2.57	72.44 ± 2.53	0.04 ± 0.04	ND
Squid:							
Splendid squid	5	Boiling	3	86.88 ± 1.47	79.90 ± 1.04	ND	ND
Prawn:							
Giant tiger prawn	5	Boiling	2.5	80.11 ± 1.31	73.94 ± 1.53	ND	ND

The reported values are mean ± SD.

ND: not detected.

4.3 Heme Iron Content Analysis

Heme iron contents of raw and cooked food of animal sources were determined by using Hornsey *et al.*, (1956) method as showed in Table 23-28.

Heme iron contents were not detected in duck and hen egg, milk and soybean milk.

Table 23 shows mean ± SD, range and median of heme iron content in raw and cooked beef, pork and liver (mg/100g wet and dry weight). Pork liver was a good source of heme iron content (2.86 mg/100g wet weight), while heme iron contents of raw and cooked beef loin were found to be 1.33 and 1.07 mg/100g, respectively. Heme iron contents of pork loin were slightly higher than pork tenderloin (0.42 and 0.30 mg/100g wet weight). After cooking, heme iron contents of cooked beef, pork and liver were significantly decreased ($p < 0.05$).

Table 24 shows mean ± SD, range and median of heme iron content in raw and cooked poultry and liver (mg/100g wet and dry weight). Heme iron contents were higher in both raw and cooked chicken drumsticks than chicken breast. While chicken liver had the highest heme iron contents (3.06 mg/100g wet weight). After cooking, heme iron contents were significantly decreased ($p < 0.05$).

Mean ± SD, range and median of heme iron contents of raw and cooked meatballs and sausages products (mg/100g wet and dry weight) varied widely as showed in Table 25 and 26. Heme iron contents of raw meatball and sausage products were slightly decreased after cooking. Among cooked meatballs and sausage products, cooked beef ball and non-smoked pork sausage provide the highest amount of heme iron.

Table 27 shows mean ± SD, range and median of heme iron content in cooked porcine and chicken blood curds (mg/100g wet and dry weight). Cooked blood curd contained very high heme iron contents. The average heme iron contents were 15.38 and 9.17 mg/100g wet weight, respectively of cooked porcine and chicken blood curd. When the heating time was increased heme iron contents in both cooked chicken and porcine blood curd slightly decreased with approximate 3 and 7%, respectively.

Table 28 shows mean ± SD, range and median of heme iron contents in raw and cooked shellfish, fish, squid and prawn (mg/100g wet and dry weight). Heme iron contents of cooked shellfish were high, ranging from 2.45 to 9.05 mg/100 g wet weight. Heme iron contents of fish were lower than shellfish but higher than prawn and squid. Heme iron contents in raw squid and prawn were found to be 0.08 and 0.12 mg/100g wet weight, respectively. After cooking, heme iron contents per wet weight of all fishes, Splendid squid and Giant tiger prawn were decreased significantly with approximate 20-65% ($p<0.05$). Among the animal food sources, shellfish especially Ark shells and Mussels provide more higher heme iron content than those from other animal food sources (except in cooked blood curds and beef loin).

Table 23 Heme iron content (mg/100 g wet and dry weight) in raw and cooked meat.

Food Items	No. of samples	Cooking method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Beef:						
Loin	13	Boiling (10 min)	1.33 ± 0.62* [1] (0.39-2.17) [2] 1.22 [3]	1.07 ± 0.53* (0.23-1.92) 0.92	5.77 ± 2.79** (1.55-9.55) 5.62	2.79 ± 1.47** (0.61-5.50) 2.29
Pork:						
Tenderloin	15	Boiling (5 min)	0.30 ± 0.11* (0.18-0.49) 0.26	0.25 ± 0.11* (0.13-0.48) 0.21	1.14 ± 0.45** (0.69-1.98) 0.94	0.74 ± 0.34** (0.35-1.41) 0.67
Loin	15	Boiling (5-7 min)	0.42 ± 0.15* (0.24-0.83) 0.40	0.30 ± 0.08* (0.20-0.46) 0.29	1.73 ± 0.67** (0.86-3.58) 1.66	0.99 ± 0.30** (0.60-1.67) 0.93
Liver	15	Boiling (8 min)	2.86 ± 0.47* (2.19-3.90) 2.80	2.30 ± 0.36* (1.46-2.94) 2.45	10.86 ± 1.97** (8.16-15.05) 10.41	7.92 ± 1.52** (4.42-10.21) 8.30

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

Table 24 Heme iron content (mg/100 g wet and dry weight) in raw and cooked poultry.

Food Items	No. of samples	Cooking method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Poultry:						
Breast	15	Boiling (5 min)	0.12 ± 0.03 [1] (0.10-0.17) [2] 0.12 [3]	0.11 ± 0.04 (0.05-0.16) 0.12	0.53 ± 0.12** (0.39-0.75) 0.48	0.37 ± 0.12** (0.18-0.57) 0.39
Drumsticks	15	Boiling (18-20 min)	0.31 ± 0.08 (0.22-0.51) 0.30	0.26 ± 0.09 (0.13-0.45) 0.24	1.27 ± 0.28** (0.96-1.89) 1.24	0.85 ± 0.29** (0.46-1.46) 0.79
Liver	15	Boiling (5-6 min)	3.06 ± 0.60* (1.97-3.91) 3.03	2.56 ± 0.65* (1.34-3.50) 2.36	13.02 ± 2.68** (8.06-17.04) 12.95	8.89 ± 3.07** (3.31-14.42) 8.28

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

Table 25 Heme iron contents (mg/100g wet and dry weight) in raw and cooked meatballs.

Food Items	No. of sample	Cooking Method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw [a]	cooked	raw [a]	cooked
Processed meat products:						
Meat balls:						
Cooked beef ball	5	Boiling (3 min)	0.33 ± 0.22 [1] (0-0.56) [2] 0.33 [3]	0.26 ± 0.18 (0-0.44) 0.23	1.31 ± 0.97 (0-2.27) 1.29	1.03 ± 0.79 (0-1.95) 0.76
Cooked chicken ball	7	Boiling (3 min)	0.13 ± 0.09 (0.01-0.26) 0.15	0.08 ± 0.08 (0-0.21) 0.10	0.33 ± 0.23 (0.02-0.59) 0.40	0.21 ± 0.19 (0-0.45) 0.32
Cooked pork ball	12	Boiling (3 min)	0.15 ± 0.16 (0-0.44) 0.16	0.12 ± 0.13 (0-0.33) 0.12	0.54 ± 0.55 (0-1.50) 0.58	0.43 ± 0.44 (0-1.15) 0.41
Cooked fish ball	11	Boiling (3 min)	0.05 ± 0.10 (0-0.32) 0	0.03 ± 0.07 (0-0.21) 0	0.24 ± 0.48 (0-1.46) 0	0.13 ± 0.31 (0-0.94) 0

[1] mean ± SD
[2] range (minimum-maximum)
[3] median
[a] already processed

Table 26 Heme iron contents (mg/100g wet and dry weight) in raw and cooked sausages.

Food Items	No. of sample	Cooking method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw [a]	cooked	raw [a]	cooked
Processed meat products: Sausages:						
Non-smoked pork sausage	6	Boiling (3 min)	0.44 ± 0.36 [1] (0.15-1.09) [2] 0.30 [3]	0.37 ± 0.33 (0.12-1.01) 0.24	1.09 ± 0.79 (0.42-2.47) 0.79	0.91 ± 0.73 (0.33-2.28) 0.63
Smoked pork sausage	6	Boiling (3 min)	0.60 ± 0.30 (0.23-0.96) 0.69	0.52 ± 0.26 (0.19-0.73) 0.65	1.48 ± 0.71 (0.61-2.29) 1.67	1.27 ±0.59 (0.51-1.79) 1.55
Non-smoked chicken sausage	5	Boiling (3 min)	0.33 ± 0.13 (0.20-0.50) 0.34	0.24 ± 0.23 (0-0.50) 0.34	0.87 ± 0.30 (0.55-1.28) 0.95	0.60 ± 0.50 (0-1.26) 0.81
Smoked chicken sausage	4	Boiling (3 min)	0.39 ± 0.09 (0.30-0.52) 0.38	0.33 ± 0.11 (0.25-0.47) 0.31	0.96 ± 0.37 (0.50-1.37) 0.98	0.84 ± 0.30 (0.58-1.22) 0.78

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
[a] already processed

Table 27 Heme iron contents (mg/100g wet and dry weight) in cooked blood curds.

Food Items	No. of sample	Heme iron contents (mg/100g wet weight)			Heme iron contents (mg/100g dry weight)		
		raw [a]	boiled (1min)	boil (3min)	raw [a]	boiled (1min)	boil (3min)
Cooked blood curd:							
Cooked porcine blood curd	15	15.38 ± 2.31 [1] (11.96-19.96) [2] 15.74 [3]	15.04 ± 1.93 (12.20-18.00) 15.25	14.78 ± 2.12 (12.21-17.52) 15.16	220.87 ± 41.51 (145.78-283.47) 221.57	184.97 ± 30.29 (131.45-230.89) 186.32	155.68 ± 18.59 (120.63-189.22) 151.43
Cooked chicken blood curd	15	9.17 ± 1.76 (6.05-11.53) 9.35	9.00 ± 1.87 (5.24-11.56) 9.10	8.71 ± 1.90 (4.83-11.71) 8.76	130.26 ± 21.92 (80.77-165.45) 135.51	110.62 ± 18.00 (72.86-148.91) 108.56	91.93 ± 19.06 (54.39-127.98) 90.34

[1] mean ± SD
[2] range (minimum-maximum)
[3] median
[a] already processed

Table 28 Heme iron content (mg/100 g wet and dry weight) in raw and cooked of selected seafood.

Food Items	No. of samples	Cooking method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Shellfish:						
Ark shells	5	Boiling (1 min)	-	9.05 ± 1.18 [1] (7.59-10.67) [2] 9.65 [3]	-	57.07 ± 11.87 (41.45-74.41) 56.82
		Boiling (3min)	-	5.31 ± 0.76 (4.27-6.37) 5.30	-	33.60 ± 2.81 (28.63-35.41) 34.80
Mussels (without shells [a])	5		5.00 ± 1.66 (3.43-7.79) 4.68	-	41.59 ± 17.13 (25.64-70.12) 37.17	-
Mussels		Steaming (10 min)	-	4.02 ± 0.82 (2.75-4.75) 3.94	-	15.12 ± 3.78 (9.54-19.99) 14.97
Baby clam	5	Steaming (7 min)	-	2.45 ± 0.66 (1.58-3.35) 2.41	-	9.61 ± 2.70 (6.05-12.81) 9.27

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
[a] Already prepared from the markets. -: Not determined

Table 28 Heme iron content (mg/100 g wet and dry weight) in raw and cooked of selected seafood (continued).

Food Items	No. of samples	Cooking method	Heme iron content (mg/100g wet weight)		Heme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Marine fish:						
Red snapper	5	Steaming (15 min)	0.36 ± 0.09*[1] (0.25-0.48)[2] 0.36[3]	0.21 ± 0.07* (0.11-0.28) 0.22	1.72 ± 0.45** (1.19-2.39) 1.68	0.79 ± 0.27** (0.39-1.06) 0.83
Fresh short-bodied mackerel	5	Steaming (2.5 min)	0.79 ± 0.42* (0.34-1.27) 0.70	0.63 ± 0.41* (0.21-1.15) 0.56	2.98 ± 1.15** (1.51-4.13) 3.38	2.09 ± 1.17** (0.81-3.35) 2.22
Steamed short-bodied mackerel	5	-	-	0.93 ± 0.47 (0.41-1.56) 1.01	-	2.76 ± 1.51 (1.08-5.03) 2.74

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.
- : Not determined

Table 28 Heme iron content (mg/100 g wet and dry weight) in raw and cooked of selected seafood (continued).

Food Items	No. of sample	Cooking method	Heme iron contents (mg/100g wet weight)		Heme iron contents (mg/100g dry weight)	
			raw	cooked	raw	cooked
Fresh water fish:						
Fresh water catfish	5	Steaming (15 min)	0.67 ± 0.18* [1] (0.35-0.77) [2] 0.74 [3]	0.50 ± 0.21* (0.27-0.70) 0.62	2.40 ± 0.59** (1.37-2.80) 2.67	1.63 ± 0.64** (0.91-2.26) 1.94
Striped snake-head fish	5	Steaming (15 min)	0.38 ± 0.17 (0.19-0.62) 0.33	0.29 ± 0.14 (0.11-0.49) 0.29	1.62 ± 0.64** (0.72-2.43) 1.64	1.06 ± 0.52** (0.37-1.80) 1.00
Nile tilapia	5	Steaming (15 min)	0.40 ± 0.14 (0.24-0.59) 0.39	0.33 ± 0.23 (0.10-0.63) 0.36	1.81 ± 0.67 (1.01-2.61) 1.87	1.23 ± 0.85 (0.36-2.35) 1.42
Squid:						
Splendid squid	5	Boiling (3 min)	0.08 ± 0.02 (0.05-0.11) 0.08	0.06 ± 0.02 (0.03-0.07) 0.07	0.61 ± 0.21** (0.33-0.90) 0.62	0.28 ± 0.11** (0.14-0.36) 0.36
Prawn:						
Giant tiger prawn	5	Boiling (2.5 min)	0.12 ± 0.06* (0.06-0.21) 0.09	0.07 ± 0.04* (0.01-0.12) 0.07	0.60 ± 0.29** (0.31-0.96) 0.47	0.27 ± 0.18** (0.04-0.46) 0.26

[1] mean ± SD, [2] range (minimum-maximum) and [3] median.

* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.

* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

- : Not determined.

4.4 Nonheme Iron Content Analysis

Nonheme iron contents of raw and cooked foods of animal sources were determined by the method of Rhee are shown in Table 29-36.

Table 29 shows mean ± SD, range and median of nonheme iron contents in individual milk and soybean milk (mg/100ml wet weight). Large variations of nonheme iron contents were found in soybean milk, pasteurized milk and ultra heat-treated milk ranged from 0.08-0.18 mg/100ml. The averages mean of nonheme iron from pasteurized and UHT were 0.12 and 0.14 mg/100ml, respectively.

Table 30 shows mean ± SD, range and median of nonheme iron content in whole egg and egg yolk of duck and hen egg (mg/100 g wet and dry weight). Nonheme iron contents of egg yolk in both duck and hen eggs were approximately twofold higher than in whole egg. After cooking, nonheme iron contents per dry weight of whole egg and egg yolk in both duck and hen eggs were decreased approximately 1-8%, but there were no significantly difference. Nonheme iron contents were not detected in egg white of duck and hen egg.

Table 31 shows mean ± SD, range and median of nonheme iron content in raw and cooked meats (mg/100g wet and dry weight). Pork liver had the highest nonheme iron contents (9.42 mg/100g wet weight), while raw and cooked beef loin were found to be 0.68 and 1.31 mg/100g wet weight, respectively. Pork loin, raw or cooked, was higher in nonheme iron contents than pork tenderloin. After cooking, nonheme iron contents expressed as dry weight of beef loin, pork loin and pork liver were significantly increased ($p<0.05$) with approximate 13% except in pork tenderloin.

Table 32 shows mean ± SD, range and median of nonheme iron content in raw and cooked poultry (mg/100g wet and dry weight). Nonheme iron contents in raw and

cooked drumsticks were about twofold higher than breast. Nonheme iron contents in chicken breast were found to be only 0.27 mg/100g wet weight. Chicken liver contained very high nonheme iron contents in raw and cooked states. After cooking nonheme iron contents of drumsticks and liver expressed as dry weight was significantly increased ($p<0.05$) except in breast.

Table 33 and 34 shows mean ± SD, range and median of nonheme iron content in raw and cooked processed meat products (mg/100g wet and dry weight). For meatball products, nonheme iron contents were low ranging from 0.67-1.13 mg/100 g wet weight. In sausages products, nonheme iron contents were the highest in processed non-smoked pork sausage (1.43 mg/100g wet weight). The lowest nonheme iron contents were found in processed non-smoked chicken sausage (0.47 mg/ 100g wet weight). After cooking, nonheme iron contents of cooked meatball and sausage products seemed to be unchanged.

Table 35 shows mean ± SD, range and median of nonheme iron content in raw and cooked porcine and chicken blood curds (mg/100g wet and dry weight). Nonheme iron contents in porcine blood curd were slightly higher than chicken blood curd approximately 22%. When the heating time was increased, nonheme iron contents in both chicken and porcine blood curd increased approximately 25%.

Table 36 shows mean ± SD, range and median of nonheme iron content in raw and cooked seafood (mg/100g wet and dry weight). Nonheme iron contents were very high in cooked shellfish especially in 3 minutes boiled Ark shells and Baby clam. In raw fish, nonheme iron contents were low, ranging from 0.66 to 1.21 mg/100g wet weight. Nonheme iron contents in raw squid only found to be 0.20 mg/100g wet weight. After cooking, nonheme iron contents in Red snapper, Fresh water fish,

Striped snake-head fish, Nile tilapia and Splendid squid (mg/100g dry weight) were increased approximately 10% but there were no statistically significant difference except in Fresh short-bodied mackerel and Giant tiger prawn.

Table 29 Nonheme iron content (mean ± SD) in milk.

Food Items	No. of sample	Nonheme iron content (mg/100 ml wet weight)
Pasteurized milk	15	0.09 ± 0.04 [1] (0.01-0.15) [2]
Pasteurized sweet milk	15	0.13 ± 0.03 (0.07-0.18)
Pasteurized chocolate milk	15	0.14 ± 0.03 (0.11-0.20)
Pasteurized strawberry milk	15	0.10 ± 0.03 (0.03-0.13)
Ultra heat-treated milk	15	0.08 ± 0.04 (0.01-0.14)
Ultra heat-treated sweet milk	15	0.17 ± 0.03 (0.12-0.22)
Ultra heat-treated chocolate milk	15	0.18 ± 0.02 (0.15-0.21)
Ultra heat-treated soybean milk	6	0.12 ± 0.02 (0.10-0.14)

[1] mean ± SD
[2] range (minimum-maximum)

Table 30 Nonheme iron content in eggs.

Food Items	No. of sample	Cooking methods	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Hen egg:						
Whole egg	15	Boiling (5 min)	1.46 ± 0.21* [1] (1.18-1.81) [2] 1.45 [3]	1.60 ± 0.13* (1.38-1.85) 1.60	6.15 ± 0.97 (4.78-7.93) 6.13	6.33 ± 0.48 (5.60-7.24) 6.21
Yolk	15	Boiling (5 min)	5.80 ± 1.02 (4.27-8.32) 5.83	6.05 ± 0.95 (4.85-8.46) 5.93	11.70 ± 2.02 (8.54-16.80) 11.52	11.85 ± 1.98 (9.28-16.84) 11.77
Duck egg						
Whole egg	15	Boiling (5 min)	2.8 ± 0.42* (2.10-3.57) 2.89	3.20 ± 0.44* (2.40-3.80) 3.20	9.56 ± 1.49 (7.15-12.26) 9.63	9.98 ± 1.52 (6.97-12.07) 10.24
Yolk	15	Boiling (5 min)	3.48 ± 0.95 (1.68-4.37) 3.77	3.89 ± 0.68 (2.39-4.68) 3.94	6.53 ± 1.79 (3.18-8.39) 7.09	7.07 ± 1.21 (4.46-8.42) 7.27

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$. .

Table 31 Nonheme iron content (mg/100 g wet and dry weight) in raw and cooked meat.

Food Items	No. of samples	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Beef:						
Loin	13	Boiling (10 min)	0.68 ± 0.30* [1] (0.14-1.04) [2] 0.73 [3]	1.31 ± 0.51* (0.55-2.16) 1.34	2.96 ± 1.38** (0.48-4.60) 3.01	3.37 ± 1.30** (1.37-5.50) 3.42
Pork:						
Tenderloin	15	Boiling (5 min)	0.44 ± 0.12* (0.27-0.70) 0.42	0.56 ± 0.11* (0.40-0.78) 0.54	1.68 ± 0.50 (1.03-2.82) 1.65	1.69 ± 0.36 (1.18-2.37) 1.68
Loin	15	Boiling (5-7 min)	0.52 ± 0.18* (0.28-0.96) 0.50	0.76 ± 0.21* (0.41-1.15) 0.80	2.17 ± 0.82** (1.12-4.14) 2.00	2.45 ± 0.73** (1.40-4.18) 2.41
Liver	15	Boiling (8 min)	9.42 ± 2.41* (5.98-13.60) 8.95	10.32 ± 2.61* (6.71-14.91) 9.88	34.96 ± 9.72** (23.07-55.37) 32.59	35.12 ± 9.72** (23.33-55.84) 33.03

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

Table 32 Nonheme iron content (mg/100 g wet and dry weight) in raw and cooked poultry.

Food Items	No. of samples	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Poultry:						
Breast	15	Boiling (5 min)	0.27 ± 0.08* [1] (0.15-0.39) [2] 0.30 [3]	0.34 ± 0.08* (0.21-0.46) 0.39	1.13 ± 0.35 (0.61-1.63) 1.20	1.14 ± 0.29 (0.71-1.50) 1.27
Drumsticks	15	Boiling (18-20 min)	0.63 ± 0.10* (0.42-0.80) 0.65	0.96 ± 0.20* (0.60-1.28) 0.95	2.62 ± 0.54** (1.55-3.43) 2.72	3.22 ± 0.74** (1.95-4.34) 3.25
Liver	15	Boiling (5-6 min)	6.74 ± 0.99* (4.83-7.83) 6.98	7.99 ± 1.31* (5.62-9.95) 8.12	28.57 ± 3.80** (21.59-33.01) 29.67	29.01 ± 3.91** (22.08-33.26) 29.95

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

Table 33 Nonheme iron contents (mg/100g wet and dry weight) in raw and cooked meatballs.

Food Items	No. of sample	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw [a]	cooked	raw [a]	cooked
Processed meat products:						
Meat balls:						
Cooked beef ball	5	Boiling (3 min)	1.13 ± 0.49 [1] (0.54-1.66) [2] 1.36 [3]	1.17 ± 0.49 (0.60-1.71) 1.36	4.45 ± 2.40 (1.82-7.53) 5.35	4.56 ± 2.35 (2.10-7.57) 5.30
Cooked chicken ball	7	Boiling (3 min)	0.69 ± 0.10 (0.52-0.81) 0.70	0.77 ± 0.13 (0.55-0.94) 0.75	1.80 ± 0.36 (1.34-2.31) 1.73	1.99 ± 0.45 (1.37-2.64) 1.94
Cooked pork ball	12	Boiling (3 min)	0.67 ± 0.25 (0.33-1.05) 0.68	0.72 ± 0.29 (0.35-1.36) 0.64	2.34 ± 0.75 (1.21-3.51) 2.50	2.43 ± 0.75 (1.31-3.76) 2.29
Cooked fish ball	11	Boiling (3 min)	0.84 ± 0.24 (0.54-1.41) 0.76	0.86 ± 0.30 (0.54-1.57) 0.83	4.70 ± 1.52 (3.29-8.13) 3.85	4.79 ± 1.84 (2.44-8.80) 4.75

[1] mean ± SD
[2] range (minimum-maximum)
[3] median
[a] already processed

Table 34 Nonheme iron contents (mg/100g wet and dry weight) in raw and cooked sausages.

Food Items	No. of sample	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw [a]	cooked	raw [a]	cooked
Processed meat products: Sausages:						
Non-smoked pork sausage	6	Boiling (3 min)	1.43 ± 0.39 [1] (0.71-1. 72) [2] 1.60 [3]	1.48 ± 0.30 (0.94-1.80) 1.50	3.77 ± 1.21 (1.76-4.83) 4.34	3.80 ± 0.87 (2.28-4.96) 3.87
Smoked pork sausage	6	Boiling (3 min)	1.09 ± 0.48 (0.59-1.67) 1.07	1.11 ± 0.59 (0.60-1.92) 0.88	2.77 ± 1.27 (1.47-4.40) 2.67	2.78 ± 1.61 (1.39-4.98) 2.06
Non-smoked chicken sausage	5	Boiling (3 min)	0.47 ± 0.12 (0.36-0.65) 0.46	0.52 ± 0.11 (0.43-0.68) 0.49	1.26 ± 0.31 (0.84-1.67) 1.25	1.36 ± 0.21 (1.19-1.71) 1.29
Smoked chicken sausage	4	Boiling (3 min)	0.62 ± 0.09 (0.52-0.73) 0.61	0.65 ± 0.27 (0.43-1.05) 0.55	1.47 ± 0.41 (0.87-1.75) 1.63	1.62 ± 0.76 (1.04-2.72) 1.36

[1] mean ± SD
[2] range (minimum-maximum)
[3] median
[a] already processed

Table 35 Nonheme iron contents (mg/100g wet and dry weight) in cooked blood curds.

Food Items	No. of sample	Nonheme iron contents (mg/100g wet weight)			Nonheme iron contents (mg/100g dry weight)		
		raw[a]	boiled (1min)	boil (3min)	raw[a]	boiled (1min)	boil (3min)
Cooked blood curd:							
Cooked porcine blood curd	15	2.63 ± 0.92 [1] (1.07-3.98) [2] 2.41 [3]	3.32 ± 0.92 (1.53-4.61) 3.41	4.40 ± 1.78 (1.60-8.17) 4.10	39.91 ± 19.30 (10.18-81.91) 39.06	43.40 ± 18.01 (15.33-83.32) 41.30	46.36 ± 17.94 (17.49-90.21) 41.85
Cooked chicken blood curd	15	2.35 ± 0.94 (1.24-4.04) 2.14	2.79 ± 1.19 (1.30-5.07) 2.43	3.36 ± 1.35 (1.68-6.24) 2.86	34.04 ± 15.47 (17.72-63.79) 29.31	34.83 ± 15.60 (18.03-65.68) 31.58	35.63 ± 14.25 (20.59-64.54) 31.75

[1] mean ± SD
[2] range (minimum-maximum)
[3] median
[a] already processed

Table 36 Nonheme iron content (mg/100 g wet and dry weight) in raw and cooked seafood.

Food Items	No. of samples	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Shellfish:						
Ark shells	5	Boiling (1 min)	-	8.68 ± 1.98 [1] (6.02-11.14) [2] 8.36 [3]	-	55.58 ± 18.98 (35.91-78.67) 54.81
		Boiling (3min)	-	12.33 ± 1.34 (10.97-14.49) 11.84	-	79.84 ± 20.76 (64.98-116.11) 74.09
Mussels (without shells [a])	5	-	7.15 ± 1.40 (5.89-9.33) 6.55	-	59.16 ± 16.76 (44.02-83.98) 55.01	-
Mussels	5	Steaming (10 min)	-	10.71 ± 1.41 (8.56-12.43) 10.68	-	39.73 ± 3.38 (36.03-43.28) 39.14
Baby clam	5	Steaming (7 min)	-	12.00 ± 2.58 (9.62-15.77) 11.89	-	46.41 ± 6.76 (36.83-54.93) 45.07

[1] mean ± SD, [2] range (minimum-maximum) and [3] median.
[a] Already prepared form the market - : Not determined.

Table 36 Nonheme iron content (mg/100 g wet and dry weight) in raw and cooked of selected seafood (continued).

Food Items	No. of samples	Cooking method	Nonheme iron content (mg/100g wet weight)		Nonheme iron content (mg/100g dry weight)	
			raw	cooked	raw	cooked
Marine fish:						
Red snapper	5	Steaming (15 min)	0.88 ± 0.16* [1] (0.66-1.01) [2] 0.97 [3]	1.21 ± 0.17 * (0.96-1.40) 1.19	4.23 ± 0.79 (3.13-4.92) 4.53	4.48 ± 0.56 (3.64-4.98) 4.50
Fresh short-bodied mackerel	5	Boiling (2.5 min)	1.21 ± 0.45* (0.73-1.84) 1.08	1.89 ± 0.25* (1.54-2.21) 1.90	4.65 ± 1.13** (3.24-5.98) 5.08	6.60 ± 0.47** (6.43-7.07) 6.54
Steamed short-bodied mackerel	5	-	-	1.44 ± 0.33 (0.92-1.80) 1.47	-	4.19 ± 0.87 (3.08-5.32) 4.05

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

Table 36 Nonheme iron content (mg/100 g wet and dry weight) in raw and cooked of selected seafood (continued).

Food Items	No. of sample	Cooking method	Nonheme iron contents (mg/100g wet weight)		Nonheme iron contents (mg/100g dry weight)	
			raw	cooked	raw	cooked
Fresh water fish:						
Fresh water catfish	5	Steaming (15 min)	1.15 ± 0.11 (1.06-1.32) 1.11	1.29 ± 0.19 (1.05-1.51) 1.26	4.18 ± 0.28 (3.91-4.58) 4.04	4.27 ± 0.84 (3.40-5.47) 3.81
Striped snake-head fish	5	Steaming (15 min)	0.66 ± 0.24* (0.43-1.03) 0.65	0.94 ± 0.17* (0.66-1.09) 0.97	2.83 ± 1.07 (1.62-4.23) 3.20	3.46 ± 0.88 (2.24-4.61) 3.28
Nile tilapia	5	Steaming (15 min)	0.68 ± 0.19* (0.46-0.91) 0.72	0.89 ± 0.23* (0.60-1.14) 0.82	3.08 ± 0.92 (2.00-4.02) 3.45	3.31 ± 0.85 (2.19-4.16) 3.23
Squid:						
Splendid squid	5	Boiling (3 min)	0.20 ± 0.09* (0.15-0.35) 0.16	0.34 ± 0.06* (0.28-0.44) 0.32	1.48 ± 0.48 (1.13-2.33) 1.32	1.69 ± 0.25 (1.43-2.03) 1.55
Prawn:						
Giant tiger prawn	5	Boiling (2.5 min)	0.42 ± 0.12* (0.27-0.57) 0.44	0.60 ± 0.12* (0.42-0.70) 0.63	2.12 ± 0.54** (1.33-2.60) 2.24	2.31 ± 0.49** (1.51-2.71) 2.31

[1] mean ± SD
[2] range (minimum-maximum)
[3] median.
* significant difference between raw and cooked (wet weight) by Mann-Whitney U test at $P < 0.05$.
* * significant difference between raw and cooked (dry weight) by Mann-Whitney U test at $P < 0.05$.

4.5 Comparison of Total Iron Values in Foods by Chemical Analysis (wet digestion) with Total Iron Calculated by the Summation of Heme and Nonheme Iron.

Table 37 to 44 shows comparison of total iron values in food from animal sources using chemical analysis (AAS) with total iron calculated from the summation of heme iron analyzed by the Hornsey method and nonheme iron determined by the Rhee method.

Total iron analyzed from wet-ashed samples was compared with the summation of heme and nonheme iron. The difference of total iron values in animal sources between chemical analysis and calculation method were less than 10%. The different values of the total iron, which obtained from the calculation and analysis as seen in this study, is unclear, probably due to the overestimation and /or underestimation of the technique analysis. Carpenter and Clark in 1995 also suggested that the analyzed total iron did not always equal to the sum of heme and nonheme iron (14).

Table 37 Comparison of total iron values (mg/ 100g wet weight) in milk using chemical analysis (AAS) with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis [a] (AAS)	Sum of heme and nonheme [b] iron	% difference
Pasteurized milk	0.09	0.09	0
Pasteurized sweet milk	0.14	0.13	7.69
Pasteurized chocolate milk	0.14	0.14	0
Pasteurized strawberry milk	0.10	0.10	0
Ultra heat treated milk	0.08	0.08	0
Ultra heat treated sweet milk	0.17	0.17	0
Ultra heat treated chocolate milk	0.18	0.18	0
Ultra heat treated soybean milk	0.12	0.12	0

[a] Each food items were pooled and analyzed in duplicates.

[b] Mean values from analyzed single sample of each food items which individually analyzed.

AAS: determined by Atomic Absorption Spectrophotometer.

Table 38 Comparison of total iron values (mg/ 100g wet weight) in eggs using Chemical analysis with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis [a] (AAS)	Sum of heme and nonheme [b] iron	% difference
Hen egg:			
Whole egg , raw	1.55 ± 0.20 [1] (1.39-1.80) [2]	1.46 ± 0.20 (1.27-1.71)	6.21 ± 3.98 (0.58-9.76)
,cooked	1.57 ± 0.15 (1.39-1.73)	1.60 ± 0.12 (1.46-1.76)	4.42 ± 3.41 (0.11-7.48)
Yolk egg, raw	5.88 ± 0.92 (4.87-7.22)	5.80 ± 0.99 (4.64-7.16)	1.94 ± 1.73 (0.84-4.96)
,cooked	6.10 ± 0.91 (5.10-7.40)	6.05 ± 0.92 (5.00-7.36)	0.94 ± 0.67 (0.54-2.00)
Duck egg:			
Whole egg, raw	3.00 ± 0.38 (2.57-3.51)	2.87 ± 0.41 (2.48-3.35)	4.58 ± 3.12 (0-8.43)
,cooked	3.28 ± 0.43 (2.76-3.67)	3.19 ± 0.40 (2.72-3.59)	2.52 ± 0.95 (1.47-3.97)
Yolk egg, raw	3.59 ± 1.04 (1.80-4.39)	3.48 ± 1.00 (1.76-4.33)	3.04 ± 1.55 (1.39-5.49)
,cooked	3.94 ± 0.62 (3.00-4.60)	3.89 ± 0.64 (2.90-4.21)	1.64 ± 1.63 (0-3.45)

[1] mean ± SD

[2] range (minimum-maximum)

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of each food items in three shops from five markets, which individually analyzed (n=15).

AAS: determined by Atomic Absorption Spectrophotometer.

Table 39 Comparison of total iron values (mg/ 100g wet weight) in meat using chemical analysis with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis [a] (AAS)	Sum of heme and nonheme iron [b]	% difference
Beef:			
Loin, raw	2.02 ± 0.82 [1] (0.63-2.65) [2]	2.06 ± 0.84 (0.69-2.87)	7.06 ± 3.25 (1.53-9.52)
, cooked	2.39 ± 0.86 (0.95-3.23)	2.48 ± 0.86 (2.43-3.12)	5.52 ± 3.28 (1.67-10.38)
Pork:			
Tenderloin, raw	0.69 ± 0.19 (0.48-0.98)	0.74 ± 0.23 (0.45-1.19)	7.18 ± 2.95 (4.11-10.42)
, cooked	0.75 ± 0.16 (0.56-1.00)	0.81 ± 0.20 (0.53-1.19)	7.47 ± 2.35 (5.19-10)
Loin, raw	0.88 ± 0.25 (0.71-1.31)	0.94 ± 0.29 (0.66-1.79)	5.88 ± 2.18 (3.37-8.45)
, cooked	1.09 ± 0.21 (0.90-1.43)	1.06 ± 0.23 (0.78-1.61)	9.01 ± 1.79 (6.67-10.59)
Liver, raw	11.67 ± 2.39 (9.20-15.60)	12.28 ± 2.55 (9.21-16.85)	5.33 ± 2.82 (0.93-7.83)
, cooked	11.95 ± 2.93 (8.84-16.70)	12.61 ± 2.59 (9.21-17.36)	6.06 ± 3.40 (2.30-9.28)

[1] mean ± SD

[2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=15).

AAS: determined by Atomic Absorption Spectrophotometer

Table 40 Comparison of total iron values (mg/ 100g wet weight) in poultry using chemical analysis with total iron calculated from the sum of heme and nonheme iron .

Food Items	Analysis [a] (AAS)	Sum of heme and nonheme iron [b]	% difference
Poultry:			
Breast, raw	0.37 ± 0.07 [1] (0.27-0.45) [2]	0.39 ± 0.08 (0.27-0.47)	5.08 ± 4.06 (0-9.52)
, cooked	0.44 ± 0.08 (0.30-0.50)	0.47 ± 0.09 (0.32-0.55)	6.81 ± 2.00 (4.55-6.82)
Drumsticks, raw	0.92 ± 0.07 (0.86-1.00)	0.94 ± 0.04 (0.91-1.00)	7.18 ± 2.74 (3.09-9.89)
, cooked	1.19 ± 0.10 (1.04-1.30)	1.16 ± 0.09 (1.05-1.25)	3.71 ± 2.02 (1.72-5.98)
Liver, raw	9.23 ± 1.40 (7.32-10.84)	9.83 ± 1.31 (7.23-11.59)	6.69 ± 3.11 (1.48-9.13)
, cooked	10.02 ± 1.40 (7.79 ± 11.36)	10.56 ± 1.58 (7.76-13.07)	6.52 ± 3.81 (0.83-10.17)

[1] mean ± SD

[2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=15).

AAS: determined by Atomic Absorption Spectrophotometer

Table 41 Comparison of total iron values (mg/ 100g wet weight) in meatballs using chemical analysis with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Processed meat products; meat ball			
Cooked beef ball, raw	1.34 ± 0.73 [1] (0.56-2.10) [2]	1.36 ± 0.76 (0.54-2.16)	2.14 ± 1.99 (0.02-4.55)
, cooked	1.32 ± 0.72 (0.56-2.05)	1.36 ± 0.71 (0.60-2.15)	5.29 ± 1.54 (4.88-7.14)
Cooked chicken ball, raw	0.77 ± 0.13 (0.61-0.90)	0.81 ± 0.15 (0.56-0.95)	4.66 ± 1.71 (2.20-6.10)
, cooked	0.81 ± 0.10 (0.70-0.93)	0.86 ± 0.17 (0.55-1.03)	4.39 ± 2.52 (1.43-7.53)
Cooked pork ball, raw	0.81 ± 0.14 (0.66-1.04)	0.82 ± 0.29 (0.48-1.45)	3.33 ± 0.99 (1.96-4.55)
, cooked	0.84 ± 0.14 (0.70-1.04)	0.84 ± 0.33 (0.48-1.69)	2.46 ± 1.65 (1.30-5.05)
Cooked fish ball, raw	0.82 ± 0.20 (0.55-1.08)	0.90 ± 0.16 (0.54-1.10)	2.65 ± 1.36 (1.35-4.44)
, cooked	0.80 ± 0.27 (0.51-1.13)	0.89 ± 0.27 (0.55-1.18)	3.33 ± 4.06 (0-9.80)

[1] mean ± SD

[2] range (minimum-maximum)

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed.

AAS: determined by Atomic Absorption Spectrophotometer.

Table 42 Comparison of total iron values (mg/ 100g wet weight) in sausages using Chemical analysis with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Processed meat products; sausage			
Non-smoked pork sausage, raw	1.70	1.87 ± 0.50 [1] (0.93-2.33) [2]	10.00
, cooked	1.93	1.86 ± 0.50 (1.13-2.69)	3.76
Smoked pork sausage, raw	1.54	1.41 ± 0.41 (0.99-1.90)	9.74
, cooked	1.52	1.63 ± 0.37 (1.22-2.12)	7.24
Non-smoked chicken sausage, raw	0.79	0.80 ± 0.22 (0.58-1.15)	1.27
, cooked	0.76	0.76 ± 0.31 (0.43-1.18)	0
Smoked chicken sausage, raw	0.95	1.00 ± 0.15 (0.82-1.16)	5.26
, cooked	0.97	0.98 ± 0.37 (0.68-1.52)	1.03

[1] mean ± SD

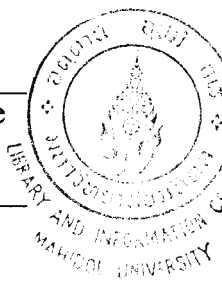
[2] range (minimum-maximum).

[a] Each food items from the same supermarket were pooled and analyzed in duplicates. Values are mean ± SD from two supermarkets.

[b] Values are shown as mean ± SD from single sample of food items in two supermarkets which individually analyzed.

AAS: determined by Atomic Absorption Spectrophotometer.

Table 43 Comparison of total iron values (mg/ 100g wet weight) in cooked blood curds using chemical analysis with total iron calculated from the sum of heme and nonheme iron.



Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Processed meat products; cooked blood curds			
Cooked porcine blood curd, raw	17.95 ± 2.69 [1] (14.51-21.53) [2]	18.03 ± 1.97 (15.85-20.21)	4.94 ± 3.23 (0.62-9.24)
, boiled (1min)	17.63 ± 2.28 (15.01-20.34)	18.36 ± 1.79 (16.49-20.76)	6.69 ± 2.14 (4.49-9.86)
, boiled (3min)	18.98 ± 1.77 (17.01-20.85)	19.18 ± 1.72 (16.88-21.23)	5.64 ± 2.89 (2.43-9.92)
Cooked chicken blood curd, raw	10.79 ± 1.91 (8-12.93)	11.52 ± 1.85 (8.66-13.44)	7.05 ± 2.27 (3.94-9.30)
, boiled (1min)	11.51 ± 1.82 (9.06-13.31)	11.79 ± 2.03 (8.77-14.07)	4.17 ± 2.07 (1.47-6.70)
, boiled (3min)	12.11 ± 2.01 (10.08-14.94)	12.06 ± 1.76 (9.49-14.23)	6.15 ± 1.39 (4.65-8.06)

[1] mean ± SD

[2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=15).

AAS: determined by Atomic Absorption Spectrophotometer.

Table 44 Comparison of total iron value (mg/ 100g wet weight) in seafood using chemical analysis with total iron calculated from the sum of heme and nonheme iron.

Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Shellfish:			
Ark shells, boiled (1min)	16.76 ± 2.15 [1] (13.70-18.86) [2]	17.73 ± 1.70 (14.98-19.54)	3.78 ± 3.37 (0.33-9.34)
, boiled (3min)	16.55 ± 1.30 (14.70-17.46)	17.64 ± 1.03 (16.08-18.76)	6.92 ± 2.89 (3.29-10.21)
Mussels , raw [c]	11.31 ± 2.67 (8.52-15.66)	12.15 ± 3.02 (9.32-17.12)	7.26 ± 2.80 (2.84-9.39)
, steamed	13.52 ± 1.27 (12.23-15.56)	14.73 ± 1.58 (13.31-17.18)	8.92 ± 3.23 (3.18-10.76)
Baby clam, steamed	13.74 ± 2.90 (10.90-18.33)	14.45 ± 3.07 (11.20-19.12)	5.03 ± 2.53 (2.75-8.87)

[1] mean ± SD

[2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=5).

[c] Already prepared from the market.

AAS: determined by Atomic Absorption Spectrophotometer.

Table 44 Comparison of total iron values (mg/ 100g wet weight) in seafood using chemical analysis with total iron calculated from the sum of heme and nonheme iron (continued) .

Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Marine fish:			
Red snapper, raw	1.18 ± 0.19 [1] (0.95-1.38) [2]	1.24 ± 0.23 (0.96-1.47)	6.58 ± 1.58 (4.17-8.46)
, steamed	1.34 ± 0.16 (1.13-1.50)	1.42 ± 0.16 (1.33-1.51)	5.70 ± 2.13 (4.14-9.02)
Fresh short-bodied mackerel, raw	1.88 ± 0.83 (0.98-3.00)	2.00 ± 0.85 (1.07-3.11)	7.35 ± 2.94 (3.67-10.16)
, boiled	2.47 ± 0.54 (1.90-3.30)	2.52 ± 0.64 (1.75-3.36)	6.77 ± 3.07 (1.82-9.63)
Steamed-shorted bodied mackerel	2.24 ± 0.65 (1.35-3.11)	2.38 ± 0.64 (1.45-3.21)	6.66 ± 3.19 (3.22-10.50)

[1] mean ± SD

[2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown as mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=5).

AAS: determined by Atomic Absorption Spectrophotometer

Table 44 Comparison of total iron values in seafood (mg/ 100g wet weight) using chemical analysis with total iron calculated from the sum of heme and nonheme iron (continued).

Food Items	Analysis (AAS) [a]	Sum of heme and nonheme iron [b]	% difference
Fresh water fish:			
Fresh water catfish, raw	1.71 ± 0.22 [1] (1.33-1.89) [2]	1.82 ± 0.23 (1.46-2.09)	6.89 ± 3.24 (2.82-10.58)
, steamed	1.68 ± 0.26 (1.28-2.00)	1.79 ± 0.28 (1.33-2.06)	6.57 ± 3.09 (3.00-9.94)
Striped snake-head fish, raw	0.97 ± 0.30 (0.58-1.42)	1.04 ± 0.32 (0.62-1.51)	8.14 ± 1.50 (6.34-10.00)
, steamed	1.22 ± 0.29 (0.80-1.51)	1.23 ± 0.30 (0.77-1.56)	4.56 ± 2.80 (2.90-9.52)
Nile tilapia, raw	1.02 ± 0.32 (0.70-1.42)	1.08 ± 0.33 (0.75-1.50)	6.15 ± 2.16 (2.78-8.57)
, steamed	1.14 ± 0.33 (0.81-1.68)	1.22 ± 0.32 (0.88-1.74)	7.12 ± 2.56 (3.57-10.28)
Squid:			
Splendid squid, raw	0.27 ± 0.07 (0.22-0.38)	0.28 ± 0.07 (0.23-0.40)	0.27 ± 0.07 (0-8.70)
, boiled	0.37 ± 0.05 (0.33-0.44)	0.40 ± 0.05 (0.35-0.47)	6.41 ± 2.23 (2.86-8.82)
Prawn:			
Giant tiger prawn, raw	0.52 ± 0.18 (0.32-0.73)	0.54 ± 0.17 (0.35-0.78)	7.67 ± 1.86 (6.25-10)
, boiled	0.65 ± 0.14 (0.50-0.80)	0.67 ± 0.15 (0.47-0.82)	5.04 ± 2.37 (2.50-7.69)

[1] mean ± SD, [2] range (minimum-maximum).

[a] Each food items from three different shops at the same market were pooled and analyzed in duplicates. Values are mean ± SD from five markets (n=5).

[b] Values are shown mean ± SD from single sample of food items in three shops from five markets, which individually analyzed (n=5).

AAS: determined by Atomic Absorption Spectrophotometer.

4.6 The Effect of Boiling and Steaming on Percent True Retention (% TR) and Percent Heme Iron Loss

The effect of boiling and steaming on percent true retention and percent heme loss are shown in Table 45-50. There was a considerable variation in %TR and % heme iron loss of cooked meats (Table 45) and poultry (Table 46), ranging from 47 to 69% and 31 to 53%, respectively. Heme iron retention (%TR) and percent heme iron loss of cooked pork liver and chicken liver (100° C, 5-8 minutes) were almost similar (62.9 and 60.3% and 37.0% and 39.6%, respectively). In addition, when comparing %TR of all meats and poultry, %TR of beef loin was the lowest with approximately 42%. The percentage of true retention (%TR) and percent heme iron loss of cooked beef ball and cooked pork ball (Table 47-48) were almost as the same as in meat sausage products (54-86% and 13-45%, respectively), except cooked fish ball was not detected. It is interesting to note that after boiling, heme iron in 1 minute boiled cooked porcine and chicken blood curd were better retained than 3 minutes boiled cooked porcine and chicken blood curd and also in other animal food sources as shown in Table 49. After cooking (boiled) %TR of squid and prawn were lower than fresh short-bodied mackerel. When comparing the effect of steaming on %TR, the results showed that fresh water catfish and striped snake-head fish (15 minutes) had better %TR and % heme iron than other types of fishes as seen in Table 50.

Table 45 Effect of cooking on the heme iron content of meats.

Food Items	Cooking Method	% True retention (wet weight)	% Heme loss [a]
Beef:			
Loin	Boiling (10 min)	47.33 ± 12.59 [1] (34.62-80.82) [2] 42.79 [3]	52.67 ± 12.59 (19.18-65.38) 57.21
Pork:			
Tenderloin	Boiling (5 min)	60.46 ± 12.01 (42.37-78.83) 64.45	39.54 ± 12.01 (21.17-57.63) 35.55
Loin	Boiling (5-7 min)	56.80 ± 14.81 (33.31-77.86) 58.54	43.20 ± 14.81 (22.14-66.69) 41.46
Liver	Boiling (8 min)	62.93 ± 12.96 (42.65-85.11) 61.52	37.07 ± 12.96 (14.89-57.35) 38.48

[1] mean ± SD

[2] range (minimum-maximum).

[3] median.

[a] 100 - % True retention

Table 46 Effect of cooking on the heme iron content of poultry.

Food Items	Cooking Method	% True retention (wet weight)	% Heme loss [a]
Poultry:			
Breast	Boiling (5 min)	68.69 ± 15.73 [1] (37.15-98.66) [2] 67.43 [3]	31.31 ± 15.73 (1.34-62.85) 32.57
Drumsticks	Boiling (18-20 min)	55.50 ± 11.08 (34.17-68.52) 60.02	44.50 ± 11.08 (31.48-65.83) 39.98
Liver	Boiling (5-6 min)	60.34 ± 7.86 (49.03-72.77) 61.54	39.66 ± 7.86 (27.23-50.97) 38.46

[1] mean ± SD

[2] range (minimum-maximum).

[3] median

[a] 100 - % True retention

Table 47 Effect of cooking on the heme iron content of meatballs.

Food Items	Cooking Method	% True retention (wet weight)	% Heme loss [a]
Processed meat products; meat ball			
Cooked beef ball	Boiling (3 min)	79.46 ± 14.65 [1] (60.11-93.92) [2] 81.91 [3]	20.54 ± 14.65 (6.08-39.89) 18.09
Cooked chicken ball	Boiling (3 min)	45.47 ± 36.93 (0-81.94) 54.45	54.53 ± 36.93 (20.58-100) 45.55
Cooked pork ball	Boiling (3 min)	79.44 ± 9.18 (63.90-92.45) 79.31	20.56 ± 9.18 (7.55-36.10) 20.69
Cooked fish ball	Boiling (3 min)	ND	ND

[1] mean ± SD

[2] range (minimum-maximum).

[3] median.

[a] 100 - % True retention

ND: not detected

Table 48 Effect of cooking on the heme iron content of sausages.

Food Items	Cooking Method	% True retention (wet weight)	% Heme loss [a]
Processed meat products; sausage			
Non-smoked pork sausage	Boiling (3 min)	82.11 ± 5.84 [1] (75.89-91.45) [2] 81.81 [3]	17.89 ± 5.84 (8.55-24.11) 18.20
Smoked pork sausage	Boiling (3 min)	85.67 ± 7.06 (74.66-95.61) 86.60	14.33 ± 7.06 (4.39-25.34) 13.40
Non-smoked chicken sausage	Boiling (3 min)	55.92 ± 51.54 (0-99.22) 81.56	44.08 ± 51.54 (0.78-100) 18.44
Smoked chicken sausage	Boiling (3 min)	82.87 ± 9.04 (81.94-90.11) 85.46	17.13 ± 9.04 (9.89-18.06) 14.54

[1] mean ± SD

[2] range (minimum-maximum).

[3] median

[a] 100 - % True retention

Table 49 Effect of cooking on the heme iron content of cooked blood curds.

Food Items	% True retention (wet weight) (boiled 1 min)	% Heme loss [a]	% True retention (wet weight) (boiled 3 min)	% Heme loss [a]
Cooked porcine blood curd	95.39 ± 3.52 [1] (85.61-98.53) [2] 96.57 [3]	4.61 ± 3.52 (1.47-14.39) 3.43	91.68 ± 7.33 (74.35-99.26) 92.09	8.32 ± 7.33 (0.74-25.65) 7.91
Cooked chicken blood curd	95.96 ± 3.53 (86.16-98.95) 96.72	4.04 ± 3.53 (1.05-13.84) 3.28	92.28 ± 5.43 (78.32-98.22) 94.19	7.72 ± 5.43 (1.78-21.68) 5.81

[1] mean ± SD

[2] range (minimum-maximum).

[3] median

[a] 100 - % True retention

Table 50 Effect of cooking on the heme iron content of cooked seafood.

Food Items	Cooking Method	% True retention (wet weight)	% Heme loss [a]
Marine fish:			
Red snapper	Steaming (15 min)	43.70 ± 13.72 [1] (21.58-53.16) [2] 51.69 [3]	56.30 ± 13.72 (46.84-78.42) 48.31
Fresh short- bodied mackerel	Boiling (2.5 min)	56.08 ± 9.37 (44.80-68.76) 56.07	43.92 ± 9.37 (31.24-55.20) 43.93
Fresh water fish:			
Fresh water catfish	Steaming (15 min)	64.69 ± 18.53 (33.09-81.98) 70.11	35.31 ± 18.53 (18.02-66.91) 29.89
Striped snake-head fish	Steaming (15 min)	69.13 ± 23.95 (36.38-91.25) 79.15	30.87 ± 23.95 (8.75-63.62) 20.85
Nile tilapia	Steaming (15 min)	53.82 ± 33.63 (16.39-87.91) 55.48	46.18 ± 33.63 (12.09-83.61) 44.52
Squid:			
Splendid squid	Boiling (3 min)	39.81 ± 13.30 (26.45-57.28) 34.63	60.20 ± 13.30 (42.72-73.55) 65.37
Prawn:			
Giant tiger prawn	Boiling (2.5 min)	37.57 ± 18.80 (10.60-63.04) 37.16	62.43 ± 18.80 (36.96-89.40) 62.84

[1] mean ± SD

[2] range (minimum-maximum).

[3] median

[a] 100 - % True retention

4.7 Effect of heating time on heme and nonheme iron.

Figure 4 and 5 shows the effect of heating time on heme and nonheme iron contents of pork loin and red snapper, respectively. When heating time was increased, heme iron content in both pork loin and Red snapper were decreased.

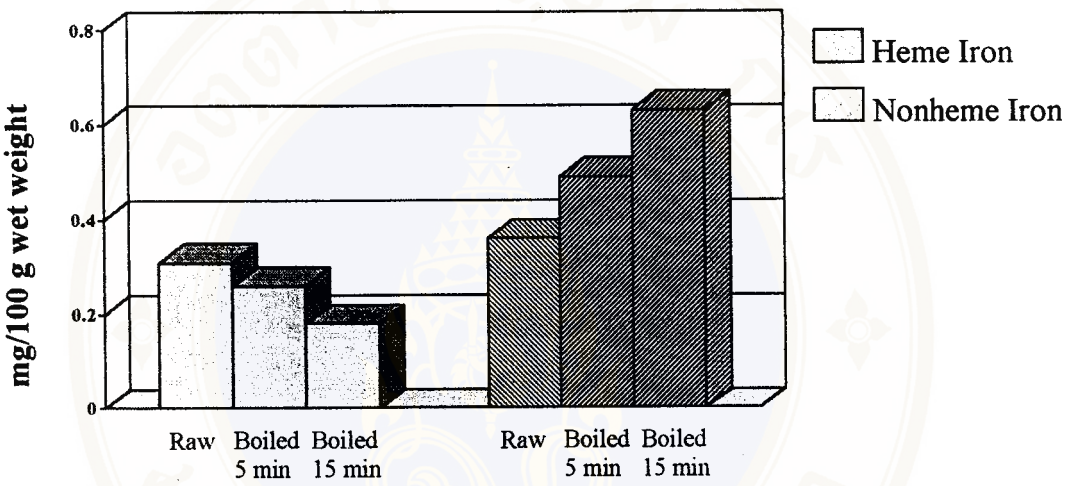


Figure 4 Effect of heating time on heme and nonheme iron contents of pork loin.

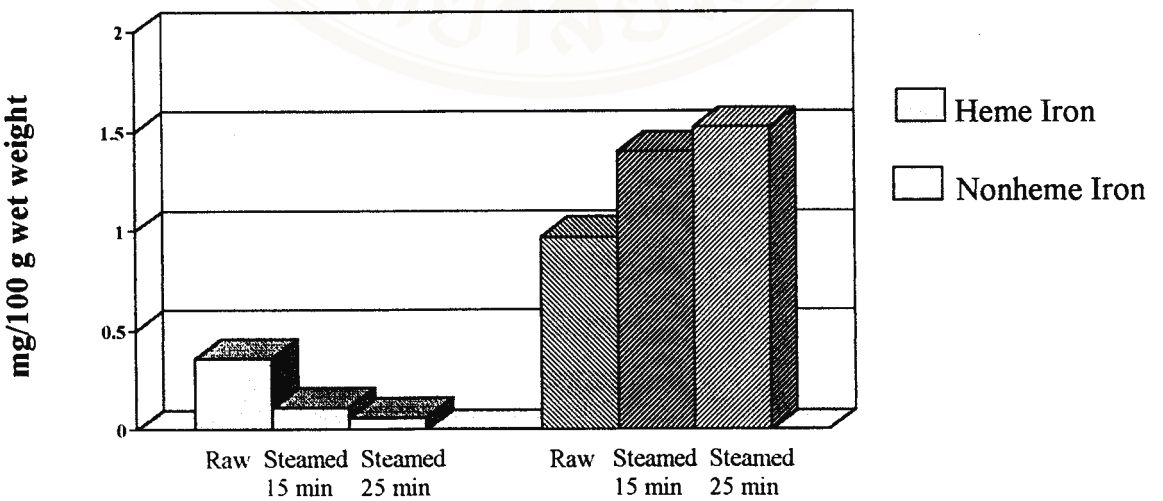


Figure 5 Effect of heating time on heme and nonheme iron contents of Red snapper.

CHAPTER V

DISCUSSION

Foods from animal sources are the main sources of dietary iron due to the presence of iron absorption enhancing factor and because the iron is in the available form of heme iron (60). In meat, the iron content is different between species and the type of tissue. In this study the results shows that heme iron content per wet weight expressed as percent of total iron, were 28-30 %, 23 %, 15-39% and 43% for cooked pork loin and cooked pork tenderloin, cooked chicken, cooked fish and cooked shellfish and cooked beef loin, respectively. The darker meats, such as beef loin, contain more of both heme and nonheme iron than paler meats, such as pork loin and tenderloin. The reason for different heme iron concentrations between dark meat and light meat is that perhaps, dark meats or red meats are well supplied with blood vessels than in light meats, which contain less myoglobin and hemoglobin. Our results were similar to those reported by Cook and Monsen (1976) that heme iron content ranged from 30 – 40% of total iron in pork (8). Carpenter et al. (1995) also estimated that the average percentage of heme iron found in cooked chicken breast is 25% which was similar to our results (14). The heme iron content of raw pork tenderloin and liver (0.30 and 2.86 mg/g wet weight) were similar to values reported by Kangsadalampai et al.(1987) (114). However, the heme iron contents of raw pork loin and raw beef loin (0.42 and 1.33 mg/100g wet weight) were lower than those reported by the same author who found 0.69 and 2.74 mg/100g wet weight, respectively. This might be due to the differences in nutritional status, genetic factors, slaughter practices, different feeding, age and sex of the animal (115). Scheeder et al. (1994) reported that the

heme iron content of veal was affected by the iron concentration in the feed (116). Sample preparation, sampling and analytical methods are also the important factors in determining contents of heme and nonheme iron (86,117).

Heme and nonheme iron content of chicken drumsticks and breast from this study were similar to the values from earlier studies (14,114). The heme iron expressed as percent of total iron of poultry was approximately 25%. It is interesting to note that the heme and nonheme iron content of drumsticks (dark muscles) were higher than in breast (light muscles). Because the dark muscles (drumsticks) are generally more active, thus having a greater blood supply than in light meat (breast) of sedentary muscles which contains less myoglobin and hemoglobin, and would be expected to have higher concentrations of iron (109,118). Nevertheless, both chicken and pork liver contained the highest percentage of nonheme iron, approximately 80%, because this organ is storage site for iron (31). For meatballs and sausage products, heme and nonheme iron content varies widely. This variation might be due to the difference in production process as well as quality and type of meat.

Interestingly, the results showed that shellfish contained higher total iron (16.13 mg/100g wet weight) and percent heme iron (32%) contents than did meat and processed meat products, except cooked blood curds had highest heme iron contents expressed as 72-82 percent of total iron (15.42 mg/100 g wet weight). Iron bioavailabilities of shellfish were expected to be higher than that meat, but not much of nutritional significance because of the infrequency which they are consumed. Chicken and porcine cooked blood curds also contained high total iron and highest percent heme iron (80%) contents. According to high total iron and highest percent

heme iron concentration in cooked blood curds, thus their bioavailability was considered to be good source of dietary iron.

Egg, although high in iron content, has long been considered to have low iron bioavailability. Most of the iron in egg is found in the yolk, in the form of nonheme iron. Egg yolk phosvitin reduces the absorption of iron when eaten with other foods (31).

In this study, a comparison of total iron values for seafood with published values of Thai Food Composition Table (1987, 1999) leads to some interesting variations (119,120). The iron values for seafood in our study are slightly higher than those values in the Thai Food Composition Table. The difference may be due to sample preparation, sample size, the method of sampling, seasonal variation, the physiological cycle and geographic location (121). The percentage of heme iron found in fish in this study is similar to values reported by Cook and Monsen (1976), who found that 30% of the total iron in Cod was heme iron (8).

In general, cooking is an important process affecting food safety, nutrition quality, palatability and digestibility and may result in some nutrient loss or physiochemical change. This study shows that cooking food of animal products decreased the amount of heme iron, which is known to be better absorbed, while the nonheme iron is increased. These findings are consisted with the observations by Igene et al., 1979; Schricker et al., 1982; Schricker and Miller 1983; King J et al., 1990 (83,85,87,122). The increase in nonheme iron content is derived from the alteration of hemoglobin and myoglobin structures. Heat treatment possibly causes porphyrin ring oxidation, chemical breakdown, iron liberation, and choleglobin formation. Iron, free from the porphyrin ring, becomes part of the nonheme iron pool

(59). The percentage of heme loss from each sample in this experiment varied between 3 to 65%, depending on the portion size, cooking time, type of meat, and the presence of oxygen. The results of this study showed that the loss of heme iron in cooked blood curds, pork loin and red snapper increased with heating time. This might be because the longer time of hemoglobin exposure to high temperature, the more porphyrin was destroyed (93) and the more denatured hemoprotein complexed. (94). The longer time of cooking also makes the texture hardened.

In addition, this experiment showed that cutting size per piece could also affect losses during the cooking process, especially in squid and prawn. This may be due to a small cutting size having an increased surface area exposure to direct heat and air relative to a larger portion; consequently; more heme degradation results. After cooking, the heme and nonheme iron content of meatballs and sausage products seem to remain unchanged, possibly due to these products containing only small amounts of heme and nonheme iron. Moreover, they passed the heating during production process, which heme proteins may be denatured (12). For these reason heme iron could not be detected.

The results in this study showed that vitamin C was not found in most of the raw and cooked food of animal sources, since they contained negligible amounts of vitamin C and considerable amounts of the vitamin C may lose during cooking. In this study a very low level of vitamin C was found in liver (2.42 mg/100 g wet weight). This result were similar to those reported by Engprasert N, 1996 and Apiradeewajeeset N, 1996, who found that vitamin C in raw liver were approximately 2.44 mg/100g wet weight (100-101). The results in this study exhibited wide variations in total iron, heme and nonheme iron contents of cooked chicken liver, cooked fish ball, cooked

beef ball, non-smoked pork sausage and milk therefore, the use of their median values for evaluating or predicting availability of iron in meal would be a good representation.

CHAPTER VI

CONCLUSION

There are two types of dietary iron based on different mechanisms of absorption: nonheme and heme iron. Nonheme iron, found in plants and animal products, has low bioavailability and is influenced greatly by a variety of enhancing and inhibiting components in the diet. Heme iron, on the other hand, is found only in animal sources, has much higher bioavailability, and is not affected by other dietary constituents. Because of these great differences in bioavailability between nonheme and heme iron and the relative quantities of dietary nonheme and heme iron must be known to accurately estimate the total amount of bioavailable iron in a food.

The objective in this study was to determine the concentration of total iron, heme and nonheme iron and vitamin C in animal products as affected by conventional method (boiling and steaming). All food samples were freshly purchased at three different shops in the same market from five local markets and two supermarkets in Bangkok and analyzed individually on the same day.

The results obtained in this study can be summarized as follows:

1. The difference of total iron values, which determined from chemical analysis and calculation was less than 10%. The analyzed total iron from chemical analysis did not equal to the results from calculation probably due to an overestimation and/ or underestimation in the techniques of analysis.

2. Animal products are not good sources of vitamin C. Results in this study showed that there were no vitamin C in milk and egg. Vitamin C contents were also not found in cooked meatball, sausage, meat and poultry, except in pork and chicken liver which tended to increase after cooking which probably results from a moisture loss.
3. Chicken and porcine cooked blood curds are the best iron sources due to its high heme iron content with approximately 80%.

In general, cooking is an important process affecting food safety, the nutrition quality, palatability and digestibility and may result in some nutrient loss or physiochemical change. This study showed that cooked foods of animal products were increased the amount of nonheme iron approximately more than 50%. In addition, long heating exposure and small pieces of food were also decreased heme iron. It can be explained that the heating destroys the heme iron molecule and releases porphyrin ring. The porphyrin ring is broken down and free iron are liberated and transformed into nonheme iron part; thus results showed that cooking increased the nonheme iron concentration. According to the results in this study, the cooking process has affected on decreasing the concentration of heme iron from foods of animal sources, however, animal products are good sources of iron, which have high bioavailability. Especially cooked blood curds, which contribute iron more than 70% of RDI per 100 g wet weight, may be good iron sources for using in fortification, supplementation in food or promotion as iron source for Thai population.

Suggestion for further studies:

Further work is needed to evaluate ...

1. The effect of stir-fry, deep fry and their cooking time on heme and nonheme iron concentration
2. Percent heme retention and heme iron loss on meats and meat products and ready to eat foods.

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APPENDIX 1

Heme iron, Total iron, Percent heme iron and Moisture in animal products

Table 51 Heme iron, Total iron, Percent heme iron and Moisture in Cooked Meat and Poultry .

Name of food	Heme iron (mg/100g wet weight)	Total iron (mg/100g wet weight)	Percent heme iron	Moisture (%)
Meat and Poultry:				
Beef loin, boiled	0.92	2.38	43.15	61.11
Pork loin, boiled	0.29	1.06	28.30	66.59
Pork tenderloin, boiled	0.25	0.81	30.86	68.87
Pork liver, boiled	2.30	12.61	18.24	68.13
Breast, boiled	0.11	0.45	23.40	70.21
Drumsticks, boiled	0.26	1.22	22.41	69.94
Chicken liver, boiled	2.56	10.56	24.24	72.50

The reported values are mean.

Table 52 Heme iron, Total iron, Percent heme iron and Moisture in Cooked Processed Meat Products .

Name of food	Heme iron (mg/100g wet weight)	Total iron (mg/100g wet weight)	Percent heme iron	Moisture (%)
Processed meat products:				
Cooked beef ball, boiled	0.26	1.43	19.12	72.85
Cooked chicken ball, boiled	0.08	0.86	9.30	60.49
Cooked pork ball, boiled	0.12	0.84	14.29	71.02
Cooked fish ball, boiled	0.03	0.89	3.37	81.73
Smoked chicken sausage, boiled	0.39	1.00	33.67	60.97
Non-smoked chicken sausage, boiled	0.24	0.76	31.58	61.74
Smoked pork sausage, boiled	0.52	1.63	31.90	59.32
Non-smoked pork sausage, boiled	0.37	1.86	19.89	60.81
Porcine blood curd, boiled 1 min.	15.04	18.07	81.92	91.73
Porcine blood curd, boiled 3 min.	14.78	18.78	77.06	90.46
Chicken blood curd, boiled 1 min.	9.00	12.06	76.34	92.11
Chicken blood curd, boiled 3 min.	8.71	12.76	72.22	91.65

The reported values are mean.

Table 53 Heme iron, Total iron, Percent heme iron and Moisture in Cooked Shellfish, Fish, Squid and Shrimp.

Name of food	Heme iron (mg/100g wet weight)	Total iron (mg/100g wet weight)	Percent heme iron	Moisture (%)
Shellfish:				
Ark shells, boiled 1 min.	9.05	17.73	51.04	83.64
Ark shells, boiled 3 min.	5.31	17.64	30.10	87.65
Mussel, steamed	4.02	14.73	27.29	73.09
Baby clam, steamed	2.45	14.45	16.96	74.27
Marine fish:				
Red snapper, steamed	0.21	1.42	14.79	73.11
Fresh short-bodied mackerel, boiled	0.63	2.52	25	71.34
Steamed short-bodied mackerel	0.93	2.38	39.08	65.59
Fresh water fish:				
Nile tilapia, steamed	0.33	1.22	27.05	73.12
Fresh water catfish, steamed	0.50	1.79	27.93	69.59
Striped snake-head fish, steamed	0.29	1.23	23.58	72.44
Squid and Shrimp:				
Splendid squid, boiled	0.06	0.40	15	79.90
Giant tiger prawn, boiled	0.07	0.67	10.45	73.94

The reported values are mean.

APPENDIX 2

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products.

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)	Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
				mean	median	mean	median	
Pasteurized milk		87.89	ND	0.09	0.09	0.09	0.09	ND
Pasteurized sweet milk		84.36	ND	0.13	0.14	0.13	0.14	ND
Pasteurized chocolate milk		83.24	ND	0.14	0.14	0.14	0.14	ND
Pasteurized strawberry milk		83.42	ND	0.10	0.11	0.10	0.11	ND
Ultra heat-treated milk		88.21	ND	0.08	0.09	0.08	0.09	ND
Ultra heat-treated sweet milk		84.60	ND	0.17	0.17	0.17	0.17	ND

Total iron values; sum of heme and nonheme iron. ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)	Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
				Mean	median	mean	median	
Ultra heat-treated chocolate milk		83.20	ND	0.18	0.18	0.18	0.18	ND
Ultra heat-treated soybean milk		84.74	ND	0.12	0.12	0.12	0.18	ND
Hen egg, whole, raw	*Gallus domesticus*	76.22	ND	1.46	1.45	1.46	1.45	ND
Hen egg, whole, boiled	*Gallus domesticus*	74.65	ND	1.60	1.60	1.60	1.60	ND
Hen egg, yolk, raw	*Gallus domesticus*	50.44	ND	5.80	5.83	5.80	5.83	ND
Hen egg, yolk, boiled	*Gallus domesticus*	48.87	ND	6.05	5.93	6.05	5.93	ND

Total iron values; sum of heme and nonheme iron.

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)	Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
				Mean	median	mean	median	
Hen egg, white, raw	*Gallus domesticus*	87.57	ND	ND	ND	-	-	ND
Hen egg, white, boiled	*Gallus domesticus*	86.94	ND	ND	ND	-	-	ND
Duck egg, whole, raw	*Anas boschas domesticus*	69.90	ND	2.80	2.89	2.80	2.89	ND
Duck egg, whole, boiled	*Anas boschas domesticus*	67.77	ND	3.20	3.20	3.20	3.20	ND
Duck egg, yolk, raw	*Anas boschas domesticus*	46.78	ND	3.48	3.77	3.48	3.77	ND
Duck egg, yolk, boiled	*Anas boschas domesticus*	45.11	ND	3.89	3.94	3.89	3.94	ND

Total iron values; sum of heme and nonheme iron

ND: Not detected. - : Not determine.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scienctific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	Median	mean	median	mean	median	
Duck egg, white, raw	*Anas boschas domesticus*	86.79	ND	ND	ND	ND	-	-	ND
Duck egg, white, boiled	*Anas boschas domesticus*	86.01	ND	ND	ND	ND	-	-	ND
Beef loin, raw	*Bos taurus*	76.42	1.33	1.22	0.68	0.73	2.00	2.13	0.07
Beef loin, boiled	*Bos taurus*	61.11	1.07	0.92	1.31	1.34	2.38	2.56	ND
Pork loin, raw	*Sus scrofa*	73.71	0.42	0.40	0.52	0.50	0.94	0.84	0.08
Pork loin, boiled	*Sus scrofa*	66.59	0.30	0.29	0.76	0.80	1.06	1.02	ND

Total iron values; sum of heme and nonheme iron.

ND: Not detected.

- : Not determined.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Pork tenderloin, raw	*Sus scrofa*	75.66	0.30	0.26	0.44	0.42	0.74	0.67	0.09
Pork tenderloin, boiled	*Sus scrofa*	68.87	0.25	0.21	0.56	0.54	0.81	0.77	ND
Pork liver, raw	*Sus scrofa*	72.94	2.86	2.80	9.42	8.95	11.83	11.31	1.84
Pork liver, boiled	*Sus scrofa*	68.13	2.30	2.45	10.32	9.88	12.61	11.85	2.24
Chicken, breast, raw	*Gallus domesticus*	76.47	0.12	0.12	0.27	0.30	0.39	0.41	0.07
Chicken, breast, boiled	*Gallus domesticus*	70.21	0.11	0.12	0.34	0.39	0.45	0.49	ND

Total iron values; sum of heme and nonheme iron

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Chicken drumsticks, raw	*Gallus domesticus*	75.53	0.31	0.30	0.63	0.65	0.94	0.93	0.08
Chicken drumsticks, boiled	*Gallus domesticus*	69.94	0.26	0.24	0.96	0.95	1.22	1.16	ND
Chicken liver, raw	*Gallus domesticus*	76.44	3.06	3.03	6.74	6.98	9.83	10.43	2.58
Chicken liver, boiled	*Gallus domesticus*	72.50	2.56	2.36	7.99	8.12	10.56	11.01	3.00
Cooked beef ball, raw	*Bos taurus*	73.20	0.33	0.33	1.13	1.36	1.45	1.72	ND
Cooked beef ball, boiled	*Bos taurus*	72.85	0.26	0.23	1.17	1.36	1.43	1.67	ND

Total iron values; sum of heme and nonheme iron

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Cooked chicken ball, raw		61.37	0.13	0.15	0.69	0.70	0.81	0.88	ND
Cooked chicken ball, boiled		60.49	0.08	0.10	0.77	0.75	0.86	0.91	ND
Cooked pork ball, raw		71.92	0.15	0.16	0.67	0.68	0.82	0.82	ND
Cooked pork ball, boiled		71.02	0.12	0.12	0.72	0.64	0.84	0.88	ND
Cooked fish ball, raw		81.73	0.05	0.00	0.84	0.76	0.89	0.82	ND
Cooked fish ball, boiled		81.73	0.03	0.00	0.86	0.83	0.89	0.85	ND

Total iron values; sum of heme and nonheme iron

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Smoked chicken sausage, raw		60.97	0.39	0.38	0.62	0.61	1.00	1.03	-
Smoked chicken sausage, boiled		59.87	0.33	0.31	0.65	0.55	0.98	0.86	-
Non-smoked chicken sausage, raw		62.66	0.33	0.34	0.47	0.46	0.80	0.78	-
Non-smoked chicken sausage, boiled		61.74	0.24	0.34	0.52	0.49	0.76	0.77	-
Smoked pork sausage, raw		60.14	0.60	0.69	1.09	1.07	1.41	1.64	-

Total iron values; sum of heme and nonheme iron

-: Not determined.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Smoked pork sausage, boiled		59.32	0.52	0.65	1.11	0.88	1.63	1.61	-
Non-smoked pork sausage, raw		61.36	0.44	0.30	1.43	1.60	1.87	1.90	-
Non-smoked pork sausage, boiled		60.81	0.37	0.24	1.48	1.50	1.86	1.86	-
Porcine blood curd, raw	*Sus scrofa*	92.80	15.38	15.74	2.63	2.41	17.98	18.41	ND
Porcine blood curd, boiled 1-min.	*Sus scrofa*	91.73	15.04	15.25	3.32	3.41	18.07	18.10	ND

Total iron values; sum of heme and nonheme iron

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin c (mg/100g)
			mean	median	mean	median	mean	median	
Porcine blood curd, boiled 3-min.	*Sus scrofa*	90.46	14.78	15.16	4.40	4.40	18.78	18.74	ND
Chicken blood curd, raw	*Gallus domesticus*	92.66	9.17	9.35	2.35	2.14	11.21	11.52	ND
Chicken blood curd, boiled 1 min	*Gallus domesticus*	92.11	9.00	9.10	2.79	2.43	12.06	11.73	ND
Chicken blood curd, Boiled 3 min	*Gallus domesticus*	91.65	8.71	8.76	3.36	2.86	12.76	11.94	ND
Ark shells, boiled 1 min	*Arca granosa*	83.64	9.05	9.65	8.68	8.36	17.73	18.01	0.18
Ark shells, boiled 3 min	*Arca granosa*	87.65	5.31	5.30	12.33	11.84	17.64	17.89	ND

Total iron values; sum of heme and nonheme iron.

ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Mussels * (without shells)	*Perna viridis*	87.65	5.00	4.68	7.15	6.55	12.15	11.23	ND
Mussels, steamed		73.09	4.02	3.94	10.71	10.68	14.73	14.52	ND
Baby clam, Steamed	*Paphia undulata*	74.27	2.45	2.41	12.00	11.89	14.45	14.07	ND
Red snapper, raw	*Lutianus*	79.14	0.36	0.36	0.88	0.97	1.24	1.33	ND
Red snapper, steamed		73.11	0.21	0.22	1.21	1.19	1.42	1.47	ND
Fresh short-bodied mackerel, raw	*Rastrelliger brachysoma*	74.37	0.79	0.70	1.21	1.08	2.00	1.78	0.06

Total iron values; sum of heme and nonheme iron.
ND: Not detected.
* Already prepared from the market.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scientific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Fresh short-bodied mackerel, boiled	*Rastrelliger brachysoma*	71.34	0.63	0.56	1.89	1.90	2.52	2.30	ND
Steamed short bodied mackerel	*Rastrelliger brachysoma*	65.59	0.93	1.01	1.44	1.47	2.38	2.39	ND
Nile tilapia, raw	*Oreochromis nilotica*	77.69	0.40	0.39	0.68	0.72	1.08	1.11	0.02
Nile tilapia, steamed	*Oreochromis nilotica*	73.12	0.33	0.36	0.89	0.82	1.22	1.18	ND
Fresh water catfish, raw	*Clarias batrachus*	72.37	0.67	0.74	1.15	1.11	1.82	1.82	ND
Fresh water catfish, steamed	*Clarias batrachus*	69.59	0.50	0.62	1.29	1.26	1.79	1.88	ND

Total iron values; sum of heme and nonheme iron.
-: ND: Not detected.

Table 54 Moisture, vitamin C, Total iron, heme and nonheme iron content of animal products (continued).

Name of Food	Scienctific name	Moisture (mg/100g)	Heme iron (mg/100g)		Nonheme iron (mg/100g)		Total iron (mg/100g)		Vitamin C (mg/100g)
			mean	median	mean	median	mean	median	
Striped snake-head fish, raw	*Channa striatus*	76.25	0.38	0.33	0.66	0.65	1.04	1.01	0.40
Striped snaked- head fish, steamed	*Channa striatus*	72.44	0.29	0.29	0.94	0.97	1.23	1.27	ND
Splendid squid, raw	*Loligo formosana*	86.88	0.08	0.08	0.20	0.16	0.28	0.25	ND
Splendid squid, boiled	*Loligo formosana*	79.90	0.06	0.07	0.34	0.32	0.40	0.37	ND
Giant tiger prawn, raw	*Penaeus monodon*	80.11	0.12	0.09	0.42	0.44	0.54	0.50	ND
Giant tiger prawn, boiled	*Penaeus monodon*	73.94	0.07	0.07	0.60	0.63	0.67	0.70	ND

Total iron values; sum of heme and nonheme iron. ND: Not detected.

APPENDIX 3

Table 55 Weight per cut, percent edible portion and percent weight loss of cooked meats and poultry.

Food Items	No. of sample	Weight per cut (g)	%Weight loss	%Edible portion
Beef:				
Loin	13	42.78 ± 0.18	42.06 ± 3.60	100
Pork:				
Tenderloin	15	49.21 ± 3.28	26.82 ± 2.96	100
Loin	15	50.94 ± 2.45	26.32 ± 2.88	100
Liver	15	40.95 ± 3.48	28.45 ± 3.66	100
Chicken:				
Breast	15	36.61 ± 2.61	24.66 ± 6.95	100
Drumsticks	15	121.16 ± 14.18	40.12 ± 8.42	67.53
Liver	15	29.85 ± 5.07	27.06 ± 3.36	100

The reported values are mean ± SD.

Table 56 Weight per piece, percent edible portion and percent weight loss of processed meat products.

Food Items	No. of sample	Weight per piece (g)	%Weight loss	%Edible portion
Processed meat products:				
Cooked beef ball	5	9.83 ± 0.68	1.29 ± 0.68	100
Cooked chicken ball	7	9.00 ± 0.02	1.63 ± 0.61	100
Cooked pork ball	12	6.90 ± 0.02	1.60 ± 0.45	100
Cooked fish ball	11	9.80 ± 0.05	3.05 ± 0.67	100
Smoked chicken sausage	4	13.56 ± 0.58	1.28 ± 0.56	100
Non-smoked chicken sausage	5	14.15 ± 1.00	1.71 ± 0.68	100
Smoked pork sausage	6	15.14 ± 0.98	2.10 ± 0.89	100
Non-smoked pork sausage	6	13.66 ± 1.11	1.60 ± 0.43	100

The reported values are mean ± SD.

Table 57 Weight per piece, percent edible portion and percent weight loss of cooked blood curds.

Food Items	No. of sample	Weight per cut(g)	% Weight loss		% Edible portion
			boiled (1 min)	boiled (3 min)	
Cooked blood curd:					
Cooked porcine blood curd	15	65.61 ± 5.23	0.22 ± 0.12	1.76 ± 0.69	100
Cooked chicken blood curd	15	64.32 ± 4.49	0.30 ± 0.18	1.74 ± 0.43	100

The reported values are mean ± SD.

Table 58 Weight per cut, percent edible portion and percent weight loss of cooked Shellfish, fish, squid and shrimp.

Food Items	No. of sample	Weight per pieces (g)	%Weight loss	% Edible portion
Shellfish:				
Ask shells	5	5.72 ± 2.33	-	40
Mussels	5	7.90 ± 1.96	70.67 ± 7.18	32.4
Baby clam	5	3.71 ± 0.84	66.58 ± 4.09	38.5
Fish:				
Marine fish :				
Red snapper	5	35.92 ± 2.82	23.32 ± 10.55	-
Fresh short-bodied mackerel	5	26.14 ± 3.86	23.16 ± 7.84	80.94
Fresh water fish				
Fresh water catfish	5	48.08 ± 7.11	13.65 ± 1.01	-
Striped snake-head Fish	5	66.67 ± 8.01	10.77 ± 1.16	-
Nile tilapia	5	35.92 ± 2.82	18.77 ± 2.61	-
Squid:				
Splendid squid	5	2.75 ± 0.72	45.37 ± 3.84	97.82
Prawn:				
Giant tiger prawn	5	3.63 ± 0.37	39.71 ± 3.85	51.40

The reported values are mean ± SD.

- Not determined.

APPENDIX 4

Determination of moisture (Air –oven method)

Principle:

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

Procedure:

1. Weigh approximately 20 g of acid washed sand into a porcelain dish containing a small glass stirring rod and dry. Dry in hot air oven at 100±5°C for 30 min.
2. Remove the sand dish and allow it to cool in desiccator.
3. Weigh sand dish (=a g) and then add approximately 5 g of sample. Reweigh (=b g)
4. Add sufficient distilled water to disperse the sample evenly and evaporate off the water as much as possible on the boiling water bath. The sample + sand should be frequently mix until drying.
5. Transfer the sample dish to hot air oven and dry the sample at 100±5°C for 2 hr.
6. Remove the sample dish and cool in a desiccator and weigh (=c g).
7. Return the sample dish to the hot air oven and dry until a constant weight is obtained. Reweigh every 30 min.

8. The different weight between each interval time should not be more than 1-3 mg.

Calculation:

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

APPENDIX 5

Determination of vitamin C

(Microfluorometric method; AOAC 1990)

Principle:

Ascorbic acid is oxidized to dehydroascorbic acid in the presented of Norit (activated charcoal). The oxidized form is reacted with 1,2 – phenylendiamin-dihydrochloride to produce a fluorophor. Fluorescent intensity is proportional to the concentration of vitamin C. The fluorescent of derivative of vitamin C is prevented by forming a H_3BO_3 dehydroascorbic acid complex prior to addition of diamine solution.

Reagents:

1. **Extract solution (prepare freshly for each analysis):** Dissolve 30 grams metaphosphoric acid (Merck # 1.00546.0500) in 80 ml of Conc. glacial acetic acid and stir until solution clear and then dilute to 1000 ml with deionized distilled water.

2. **Color reagent:** Dissolve 0.10 gram 1, 2- phenylendiamine-dihydrochloride (Fluka # 78440) in 500 ml deionized distilled water.

3. **50% Sodium acetate solution:** Dissolve 50 grams sodium acetate trihydrate (Merck # 1.06267) in 100 ml deionized distilled water.

4. **Blank solution (prepare freshly for each analysis):** Dissolve 3 grams Boric acid (Merck #1.00165.1000) in 100 ml of 50% sodium acetate.

Apparatus:

1. Spectrofluorophotometer (Shimadzu ™ ; Model RF-540)
2. Vortex mixer
3. Automatic pipette
4. Analytical balance

Procedure:

1. Weigh 5 – 10 g of homogenous sample into 250 ml beaker
2. Add 30 ml extract solution, the sample was mixed well with Altra-turrax
3. Pour the sample to 100 ml volumetric flask and dilute to 100 ml with extract solution.
4. Transfer the sample to 250 ml Erlenmeyer flask which contained 2 gram Norit (activated charcoal: Norit; Fluka # 05120) and mix well and then the sample was filtrated by filter paper (Whatman # 42).
5. Pipette 5 ml sample solution and standard solution to 100 ml volumetric flask which contained 5 ml of 50 % sodium acetate solution and dilute to 100 ml with deionized distilled water and keep in dark for 15 min at room temperature.
6. Pipette 2 ml sample solution, 2 ml standard solution and 2 ml blank solution* into test tube
7. Add 5 ml of color reagent and mix well
8. Keep in dark at room temperature for 35 min.
9. Measure fluorescent intensity using Fluorescence spectrophotometer at Excitation wavelength 350 nm and Emission wavelength 430 nm.

Note*:

Sample blank preparation: Pipette 5 ml sample solution to test tube which contained 5 ml blank solution and mix well.

Calculation:

$$\text{Vitamin C (mg/100g)} = \frac{(\text{reading sample} - \text{reading blank}) \times \text{Conc. Standard} \times \text{Vol. Dilution} \times 100}{(\text{reading standard} - \text{reading blank}) \times \text{weight of sample} \times 1000}$$

APPENDIX 6

Determination of heme iron

Principle:

This procedure is based on the extraction of hematin from foods in 80% acetone/20 % water solution. The moisture content of the sample is taken into account. It is due to the final concentration of acetone in the extract solution must be 80% acetone 20% water. Realize that the water (moisture) in the sample will add to the final volume of extract and will dilute the acetone concentration, thus the percent of moisture must be determined before analyzed of heme iron.

For example:

If, we used a 4 g of sample that has 60% moisture, it means the total water (moisture) in the sample is $4 \times 0.6 = 2.4$ g of water. Therefore a 2.4 g of water in the sample will add to total volume of the extract solution. If this sample is to be diluted to a final volume of about 25 ml.

The calculation =

Total volume (25ml) – acetone- HCl – (4.5 ml of water – water in the sample)

acetone = 25 ml

conc. HCl = 0.5 ml

water = 4.5 - 2.4 = 2.1 ml

The final extract solution must contain 80 % acetone, 18 % water and 2 % conc. HCl.

Reagents:

1. Acetone (J.T. Baker # 9006-03)
2. Concentrate hydrochloric acid (Merck # 1.00317.2500)
3. Deionized distilled water
4. Hematin from bovine blood (Sigma # H-3505)

Apparatus:

1. Polypropylene centrifuge tubes with covers (stopper) size 50ml.
2. Test tubes
3. Filter paper # 42 and funnels
4. Centrifuge (Hitachi, Himac CR5BZ)
5. Spectrophotometer (Unicam ™)

Procedures:

1. Weigh 1 to 5 g of homogenous samples (depending on expected heme iron content).
2. Add 0.5 – 2 ml conc. HCl and x X ml deionized distrilled water and mix well with mixer.
3. Add 20 – 80 ml cool acetone and mix well.
4. Transfer the sample solution into water ultrasonic bath for 10 min.
5. Keep in dark for 1 hr.

6. Take the sample solution into centrifugation at, 3000 *rpm*, 10 ° C for 10 min.
7. Determine OD of the supernatant by Spectrophotometer at 640 nm.

Standard preparation:

Stock standard hematin at Conc. 1 mg/ml:

Hematin from bovine blood (Sigma # H-3505) was accurately weighed 0.01 g and dissolved to 10 ml with 80% acetone, 18% deionized distilled water and 2% Conc. HCl.

Intermediate 100 µg/ml:

Dilute 1 ml of stock standard to 10 ml with 80% acetone, 18% deionized distilled water and 2% Conc. HCl.

Working standard: Concentration from 6.25 to 50 µg/ml

Prepare by an appropriate dilution of the intermediate standard with 80% acetone, 18% deionized distilled water and 2% Conc. HCl.

Calculation: Heme iron (µg/g)

$$\frac{\text{OD} \times \text{std. curve} \times \text{dilution} \times 0.0882}{\text{Weight of sample}}$$

The conversion factor is 1 µg of hematin equal to 0.0882 µg of heme iron/g of sample.

APPENDIX 7

Determination of nonheme iron

Principle:

Nonheme Iron assay was determined by the method of Rhee K.S. et al., 1987. The homogenous sample was extracted with acid mixture and incubated in shaking water bath at 65 ° C for 20 hrs. The determination of nonheme iron content in an aliquot of supernatant was developed with the color reagent and measured by Spectrophotometer at 540 nm.

Reagents:

1. Acid mixture: Mix HCl (6N) (Merck # 1.00317.2500) and 40% trichloroacetic acid (TCA) (Merck# 1.00807.0250) in equal volumes.
2. Bathophenantroline disulfonate: Bathophenantroline disulfonic acid (sodium salt, Sigma # B-1375), 0.162 g, dissolve in 100 ml water and add 1 ml thioglycolic acid (Sigma # T-3758). Store the reagent in refrigerator not more than 2 weeks.
3. Saturated sodium acetate solution: Sodium acetate (400g) (GR, Merck # 1.0626.7), stir with 500 ml deionized distilled water until sodium acetate undissolves.
4. Color reagent: Water: saturated sodium acetate solution: bathophenantroline disulfonate at ratio 20:20:1
5. Iron standard (Merck # 1.09972): The iron stock standard was diluted with acid-mixture from 0.5-5.0 μg Fe/ml.
6. $NaNO_2$ reagent (Mallinekrodt # G-7824): $NaNO_2$ solution, 0.39% (w/v) was prepared with water fresh each day.

7. Reagent blank: 1 ml acid-mixture plus 5 ml color reagent.

Apparatus:

1. Test tube with stopper
2. Centrifuge (Hitachi; Himac CR5BZ)
3. Shaker- water bath (Precision ™)
4. Mixer
5. Spectrophotometer (Unicam Heλios Alpha & Beta)

Procedure:

1. Weigh 2-5 g of the homogeneous sample into tube.
2. Add 15 ml acid- mixture and 0.2 ml of 0.39% $NaNO_2$ into the sample tube and loosely stoppered.
3. Incubate at 65°C in bath-shaker and shaking about 18-20 hour.
4. Cool at room temperature.
5. Centrifuge at 2500 rpm at 4° C for 10 minutes.
6. Pipette 1 ml of supernatant to test tubes and add 5 ml color reagent and mix well.
7. Keep in dark for 10 min, after 10 min read the absorbance at 540 nm.

Calculation: (Nonheme iron µg/g)

$$\text{Nonheme Iron} = \frac{\text{OD} \times \text{standard curve} \times (15 + 0.2 + \text{moisture content mg/g})}{\text{Weight of sample (g)}}$$

APPENDIX 8

Determination on total iron

Principle:

Wet ashing technique was used to prepare samples for the determination of total iron. The samples were digested by nitric acid and perchloric acid at ratio 5:1 and then determined the iron content using an atomic absorption spectrophotometer at a wavelength of 248.3 nm.

Reagents:

1. Conc nitric acid (Merk # 1.00456.2500)
2. Conc perchloric acid (JT Baker # 9652-04)
3. Ferric standard solution (Merck # 1.09972): Stock ferric nitrate standard solution (1000 ppm).
4. Intermediate standard: Dilute 10 ml of stock standard to 100 ml with deionized water to make 0.1 mg Fe/ ml standard solution.
5. Working standard: 0.5-2.0 mg Fe/100ml. Prepare by an appropriate dilution of the intermediate standard with deionized water, in the presence of 10 % 4N nitric acid.

Apparatus:

1. Teflon
2. Volumetric flask
3. Filter paper # 42 and funnels
4. Atomic absorption spectrophotometer (Varian; Spectr AA-20)

Procedure:

1. Weigh 1-5 g of the homogeneous sample (depending on expected iron content) into Teflon.
2. Add 5 ml. Conc. nitric acid and 1 ml perchloric acid to each of teflon sample and then tightly covered with lids.
3. Keep the teflon sample under fumehood at room temperature for predigestion overnight.
4. Place the teflon sample in hot air oven for 16-20 hrs or until the solution clear.
5. Transfer the digested sample to an appropriate volume of volumetric flask and dilute with deionized distilled water.
6. Measure the diluted sample, working standard iron and reagent blank by Atomic Absorption Spectrophotometer.

The instrument setting:

Flame air/ acetylene

Lamp current 5 mA

Spectral band pass 0.5 nm

Wavelength 248.3 nm

Flame stoichiometry oxidizing

Calculation:

$$\text{Total iron (mg/100 g)} = \frac{\text{obsorbance} \times \text{standard curve} \times 100}{\text{weight of sample (g)}}$$

BIOGRAPHY

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