CHAPTER I

INTRODUCTION

Iron is one of the essential elements present in small quantities in human body. Total amount of body iron varies with body weight, hemoglobin concentration, sex and size of storage compartment (Guthrie, 1975; Goodhart and Shills, 1980). Generally, the iron content in adult male and female are about 40-50 and 35-50 milligrams per kilogram body weight, respectively (Wilson et al., 1975).

Body iron is divided into two funtional compartments. An essential compartment, comprising about 70% of the total, is in hemoglobin, myoglobin and certain respiratory enzymes which catalyze oxidation-reduction processes within cell. The remainder, nonessential storage iron, is found predominantly in liver, spleen and bone marrow as ferritin and hemosiderin (Guthrie, 1975; Bothwell et al., 1979). If the total body iron is approximately 4 gm, quantitative distribution of the essential fraction is as followed: 2.5-3.0 gm (60-75%) in hemoglobin of erythrocytes, 150 mg (4%) in muscle as myoglobin, 300 mg (8%) distributed among all the cells as heme enzymes (cytochromes, cytochrome oxidase, peroxidase, catalase, etc.) or serving as a cofactor in other enzyme systems and 4 mg (0.1%) bound to transferrin as transport iron in plasma. About 1 gm (25%) is stored as ferritin

and hemosiderin (Wilson et al., 1975). While the amount of storage iron is variable, the essential body iron content is proportional to lean body mass (Pike and Brown, 1984).

Iron is responsible for ability of both hemoglobin and myoglobin which act as carriers of oxygen needed for cellular respiration. Hemoglobin carries oxygen from lung to tissues and carries carbon dioxide away from cells to the lung (Guthrie, 1975; Wilson et al., 1975). Myoglobin serves as a cell reservoir of oxygen within muscle cells (Krimlich et al., 1980; Williams, 1981). Less than 0.3% of total body iron is present in all cell as respiratory enzymes, as well as cytochromes. These enzymes transfer electrons through alternate oxidation and reduction of iron in the respiratory system. Furthermore, other enzymes such as catalase, xanthine oxidase, phenylalanine hydroxylase and peroxidase also contain iron (Pike and Brown, 1984).

Iron occurs in food primarily as ferric iron (Fe^{3+}), although some ferrous iron (Fe^{2+}) has also been found (Guthrie, 1975; Williams, 1981). Acidity of stomach contents facilitates reduction of iron to ferrous state before being absorbed (Wilson, 1975). The absorption of iron in duodenum is regulated to a certain amount, but a progressively decreasing amount is absorbed in jejunum and ileum (Brown, 1963). The absorbed iron is carried into intestinal mucosal cells where it combines with a protein,

apoferritin, to form ferritin (Bothwell et al., 1979). According to the Mucosal Block Theory, the factor that chiefly controls the absorption or rejection of ingested iron is the amount of ferritin already presents in the intestinal mucosa (Wilson et al., 1975; Pike and Brown, 1984). High level of ferritin in the cells blocks absorption of iron whereas the low level permits iron to enter. When all available apoferritin has been bound to iron, excess iron is rejected and then excreted in feces (Williams, 1981).

Mucosal ferritin delivers ferrous iron to blood system. The iron is converted back to ferric state by oxidation (Bates and Schlabach, 1973) and bound to a specific iron-binding β-globulin called transferrin or siderophilin, which serves a dual function in the transport process (Laurell, 1951). It accepts iron from intestinal tract and sites of storage or hemoglobin destruction, then delivers this iron to bone marrow for hemoglobin synthesis, to storage sites, to placenta for fetal needs and to all cells for iron containing enzymes (Goodhart and Shills, 1980). Concentration of transferrin is influenced by availability of body iron stores and by rate of erythropoiesis (Bothwell et al., 1979). Ability of transferrin to bind iron is designated as total iron-binding capacity (TIBC) of serum (Davidson et al., 1979). Among individuals with adequate iron status, serum transferrin is only 30-40% saturated with iron, depletion

of iron stores results in saturation falling to less than 15% (Bothwell et al., 1979; Pike and Brown, 1984).

Majority of transferrin iron is delivered to bone marrow where iron is released for heme synthesis (Conrad and Barton, 1981; Pike and Brown, 1984). Once the iron is incorporated into hemoglobin, it remains intact within erythrocyte for 120-day life-span until the hemoglobin is degraded in reticuloendothelial cells. The iron, when released into the plasma, is again bound to transferrin for delivery to bone marrow for heme synthesis (Conrad and Barton, 1981; Williams, 1981). Approximately 90% of the iron in new hemoglobin is obtained from such recycling (Williams, 1981). The major flow of iron in the body is unidirectional. However, in certain tissues such as liver parenchyma and intestinal mucosa, iron flow is bidirectional due to its loss in liver secretion and sloughed off epithelial cells, but this accounts for only 1-2 mg of iron daily (Conrad and Barton, 1981).

Excess of needed iron is stored as ferritin and hemosiderin. The major sites of storage are liver, spleen and bone marrow, and lesser amounts are stored in other tissues (Bothwell et al., 1979). When normal amounts of iron are stored, ferritin appears to predominate; when larger amount accumulates, the storage form is chiefly hemosiderin (Wilson et al., 1975; Williams, 1981). Both forms are capable of being mobilized for hemoglobin synthesis when the essential need for iron exists (Moore

and Dubach, 1962).

The body has a limited capacity to excrete iron (Conrad and Barton, 1981). Generally, daily total iron loss in adult male is 0.9-1.0 milligram, made up of about 0.6 milligram in feces, 0.2-0.3 milligram from skin and less than 0.1 milligram in urine (Bothwell et al., 1979). A normal female of childbearing age loses an additional 0.8-2.6 milligrams per day from menstrual bleeding (Pike and Brown, 1984) and about 500 milligrams with each pregnancy (Conrad and Barton, 1981). This results in iron deficiency unless each of these losses is balanced by absorption of an equivalent amount of iron. In normal iron repleted adult, the loss is balanced by absorption of dietary iron (1-2 mg daily) in order to maintain a relatively constant amount of body iron throughout life.

Iron deficiency is one of the most prevalent nutritional disorders in the world. It occurs almost in children and women during the childbearing years due to growth requirement of iron and iron loss from menstruation and birth (Guthrie, 1975; Conrad and Barton, 1981). In the early stage of iron deficiency, iron is released from stores. Once the stores are depleted, if further iron depletion continues, a fall in plasma iron occurs (Guthrie, 1975) and the iron-deficiency anemia is developed. Certain symptoms often observed in iron-deficient subjects are headache, fatigue, changes in appetite, vasomotor disturbance, mucosal dysfunction,

dyspnea, palpitation, gastric mucosal changes as well as progressive changes in fingernails from brittleness to thinning, flattening and eventual concaving (Conrad and Barton, 1981). The functional disturbances that are attributed to diminution of iron enzymes in iron deficiency are intelligence quotient, growth, development, working capacity and susceptibility to infection (Bothwell et al., 1979; Goodhart and Shills, 1980; Pike and Brown, 1984). Estimation of iron need can be made by determining body loss and iron intake that can replace this loss. The total excretion of iron is about 1 milligram per day. An additional 0.8 milligram is assumed to be needed by women to compensate for menstrual loss. Since the amount of iron absorbed from food is believed to be 10%, 10 milligrams is recommended for adult men and for women after menopause and 18 milligrams for women of childbearing age allows for 1.8 milligrams absorbable iron (Eschleman, 1984).

Food iron is considered to consist of two pools, heme iron and nonheme iron (Cook et al., 1972; Hallberg and Bjorn-Rasmussen, 1972).

A. Heme Iron Pool

Heme, an iron porphyrin, is found in hemoglobin and myoglobin, and accounts for nearly 40% of iron present in animal tissue (Monsen et al., 1978). Heme iron is absorbed directly as the intact iron porphyrin complex (Conrad et al., 1967; Weintraub et al., 1968) and

the heme iron is released and enters the same pool as nonheme iron within the intestinal mucosal cells (Callender et al., 1957; Turnbull et al., 1962; Layrisse and Martinez-Torres, 1972). Heme oxygenase is thought to be the enzyme responsible for releasing the iron from heme (Raffin et al., 1974). Although heme iron is only 10-15% of total daily iron intake, it provides nearly one-third of the iron absorbed each day from a mixed diet due to its high bioavailability (Bothwell et al., 1979).

The absorption of heme iron is minimally affected by composition of the diet (Hussain et al., 1965; Layrisse et al., 1968; Hallberg, 1981). However, iron is poorly absorbed from purified heme when compared to hemoglobin. It is due to the formation of macromolecular polymer (Turnbull et al., 1962; Conrad et al., 1967). It has been shown that globin maintains heme in an absorbable monomeric state (Conrad et al., 1967; Weintraub et al., 1968). Heme iron absorption is influenced by iron status. The absorption ranges from 15% for iron repleted man to 35% for those lacking iron stores (Monsen et al., 1978).

An excellent source of heme iron is meat due to its high contents of myoglobin and hemoglobin. Meat can be classified as red and white meat based principally on their color intensity. Differences in the color of meat are mainly due to differences in concentration of myoglobin which accounts for approximately three-fourths of total pigment in red meat and the remainder due to

hemoglobin of blood. Myoglobin quantity in muscle varies with species, age, sex and physical activity (Forrest et al., 1975). The muscles of immature animals have a lower myoglobin content than those of more mature individuals. The intact male has muscles that contain more myoglobin than do those of the female at comparable ages. The more exercised muscles tend to be deeper in color. This is partially because of the effect of a higher level of physical activity on their myoglobin content.

Molecules of myoglobin and hemoglobin contain a iron porphyrin compound called heme. Heme is made of four pyrrole groups united to form a porphyrin ring as shown in Figure 1.

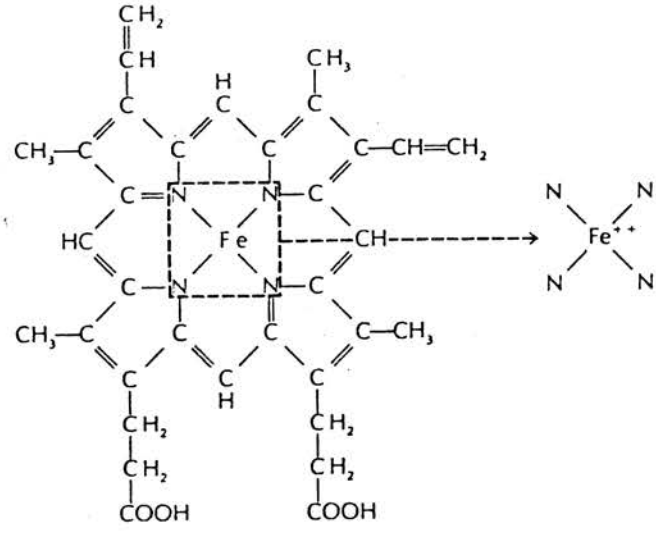

Figure 1. Formula for Heme (Charley, 1982)

Resonance of conjugated double bond in the porphyrin ring give rise to the color of meat pigments. In the center of porphyrin ring is an atom of iron linked covalently to nitrogens of the four pyrrole groups. And nitrogen of globin, a protein moiety in myoglobin molecule, is attached to the ferrous iron at the fifth coordination site.

In living tissue, myoglobin and hemoglobin exist in equilibrium between the purplish red reduced form, myoglobin and hemoglobin, and the bright cherry red oxygenated form, oxymyoglobin and oxyhemoglobin (Krimlich et al., 1980). Upon death, the oxygen in tissues is rapidly depleted, leaving the pigment in unoxygenated form with the iron in ferrous state. After exposure to air, the pigment in the cut surface of meat becomes oxygenated to form oxymyoglobin. But exposed to low levels of oxygen, the oxygen-myoglobin complex dissociates, the iron is oxidized to ferric state, and brownish red metmyoglobin is the result (Charley, 1982), as shown in Figure 2.

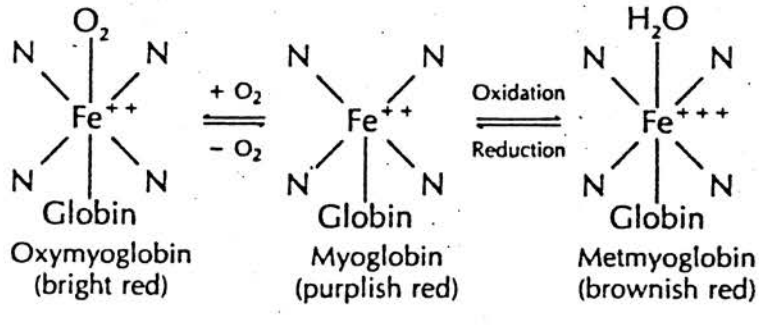

Figure 2. Pigment in Fresh Meat (Charley, 1982)

Reducing conditions which continue for a time in the meat will convert any myoglobin formed back to myoglobin.

B. Nonheme Iron Pool

Nonheme iron comes from vegetables, fruits, cereal grains, eggs and the remaining 60% in animal tissue which is mostly in storage forms of ferritin and hemosiderin (Morck and Cook, 1981). Only soluble form of nonheme iron is absorbed when it reaches the upper small intestinal mucosa. Absorption of nonheme iron is facilitated by acidity and compounds which improve solubility by converting ferric iron to ferrous state or forming loosely bound chelates (Lee and Clydesdale, 1979 a). The absorption of ferric salt is half less than that of ferrous salt. The difference can probably be ascribed to the poor solubility of ferric iron when the pH rises above 3 (Brise and Hallberg, 1962). The availability of nonheme iron is greatly influenced by a variety of enhancing and inhibiting substances present in food (Layrisse et al., 1968; Bjorn-Rasmussen et al., 1974; Cook and Monsen, 1976 a). If a meal is composed of foods low in enhancing substances or high in inhibiting substances, nonheme iron availability from that meal may be low (Sayers et al., 1973). The bioavailability of iron from this pool is the net effect of the number of dietary factors (Hallberg, 1981).

The enhancing and inhibiting iron absorption substances have been studied most exclusively in human.

Dietary factors which increase the absorption of nonheme iron, as much as four folds, are ascorbic acid (Sayers et al., 1973; Cook and Monsen, 1977) and a "meat factor" presents in meat, poultry and fish (Cook and Monsen, 1976 b). The release of amino acids and polypeptides in the upper small intestine may concern to this enhancing effect (Hussain et al., 1965; Layrisse et al., 1968). As the quantities of these substances in a complex meal increase, iron absorption increases. Beef, pork, chicken, fish and liver substantially raise rate of nonheme iron absorption but milk, cheese and eggs decrease iron availability Callender et al., 1970). Some enhancing substances such as certain sugars, amino acids and amines released during enzymatic digestion improve iron availability by decreasing both precipitation and polymerization of iron in aqueous solutions (Van Campen and Gross, 1969; Van Campen, 1973). However, those sugars and amino acids must be present in concentrations more than 100-fold greater (Charley et al., 1963). Substances which form chelates with iron can promote absorption by forming soluble monomeric complexes with iron and thus prevent its precipitation and polymerization (Loria et al., 1962; Jacobs and Miles, 1969).

It is also apparent that a group of other substances decrease nonheme iron absorption: tannic acid, phosvitin of egg yolk, phytates, calcium and phosphate salts, oxalates, carbonates and ethylenediaminetetraacetic

acid (EDTA). Although EDTA is a chelating agent, it form poorly absorbed insoluble iron precipitates and macromolecules (Disler et al., 1975; Monsen and Cook, 1976; Rossander et al., 1979; Cook et al.,1981). Because of the extensive distribution of inhibiting substances in foods, nonheme iron can be assumed to be absorbed at a low rate unless enhancing substances are present (Monsen and Balintfy, 1982). Even though the percent absorption of nonheme iron is considerably below that of heme iron (Hussain et al., 1965), the quantity of nonheme iron in the diet is many fold above that of heme iron, thus, in most meals the major contribution of available iron is made by nonheme iron (Monsen et al., 1978).

The absorption of nonheme iron depends not only on the general composition of the meal, but is further affected by the iron status of the individual (Monsen et al., 1978; Morck and Cook, 1981). Individuals with high levels of stored iron absorb smaller percentages, while iron depleted individuals absorb higher percentages of nonheme iron. The absorption can range from 2% for iron repleted man consuming a low availability meal up to 20% for the man with no or low iron stores consuming a high availability meal (Monsen et al., 1978).

One of the most significant factors in the etiology of iron deficiency is low iron availability. In evaluating causes of iron deficiency, the important consideration is not the total amount of iron ingested

but, rather, the amount of iron available for absorption (Monsen and Balintfy, 1982). The model proposed to estimate the quantity of absorbable iron in a given meal is based upon the amount of heme iron and its availability, and the amount of nonheme iron and its availability as influenced by concentrations of enhancing factors in the meal (Monsen et al., 1978; Monsen and Balintfy, 1982). Therefore, in calculating the amount of iron absorbed from different meals, the amounts of heme and nonheme iron ingested must be considered separately. And only the amounts of two enhancing substances, ascorbic acid and animal tissue, have been sufficiently defined on a quantitative basis (Monsen et al., 1978). In order to predict iron availability accurately, good estimates of heme and nonheme iron content of food are needed (Schricker et al., 1982) and influence of processing and cooking methods upon heme and nonheme iron levels is also counted (Schricker and Miller, 1983).

Total iron and heme iron contents in animal tissues are known to vary by species. Schricker et al. (1982) reported that heme iron content in pork, lamb and beef were 4.9, 9.4 and 16.2 μg Fe/gm wet weight, respectively. And the contents of heme iron, expressed as percent of total iron, were 49, 57 and 62% for pork, lamb and beef, respectively. Heme iron content in muscle also varies by age and anatomical location. Field et al. (1980) reported that myoglobin and iron contents of muscle

in steers and cows were double and triple when the values were compared with those of veal. Schricker et al. (1982) showed the differences in heme iron content among different muscles of the same animal. Processing and cooking methods had been shown to alter heme and nonheme iron levels. Igene et al. (1979) showed heating effect on beef pigment extract. They found that nonheme iron concentration in meat pigment extract was increased from 8.7% to 27.0% of total iron after cooking in boiling water to an internal temperature of 70 ^{o}C. Schricker et al. (1982) showed the similar heating effect in beef and red blood cell. Nonheme iron contents of beef and red blood cell were increased 111% and 53% after heating at 100 ^{o}C for 20 minutes, respectively.

Schricker and Miller (1983) found that addition of ascorbic acid resulted in a significant increase in nonheme iron content of beef heated at 37 ^{o}C for 24 hours. They also found that a strong oxidizing agent, hydrogen peroxide, elevated nonheme iron content of beef. The increases in nonheme iron concentrations were apparently resulted from degradation of heme from meat myoglobin and hemoglobin. Consequently, iron was released from heme complex. Although the mechanism of iron liberation had not been determined, oxidative cleavage of the porphyrin ring was suggested to involve (Schricker et al., 1982). Conversion of heme to nonheme iron caused by heat or chemical processing can reduce the availability of meat

iron (Schricker and Miller, 1983).

In addition to the form of iron, solubility of iron is another factor that affects iron absorption (Lee and Clydesdale, 1979 a). It appears that before iron can be absorbed in the gut, it must be in a solution. The more soluble iron is the better it is absorbed. Narasiga Rao and Prabhavathi (1978) had proposed an *in vitro* method for determination of available iron based on the amount of iron released at pH 7.5 after treatment with pepsin and pancreatin. The iron content was determined by α,α′-dipyridyl method. However, Lee and Clydesdale (1979 b) suggested another method for determination of soluble iron by measuring total iron content of water extract of sample with atomic absorption spectrophotometry. If this method is applied to determine soluble iron content of meat, total iron iron in water extract will be consisted of heme and nonheme iron due to their partial solubilities in water. Solubilities of various iron compounds had been shown to be correlated with their bioavailabilities (Turnbull, 1974). Consequently, alteration of soluble iron content may affect the availability of iron (Lee and Clydesdale, 1979 a & 1979 b).

Since iron contents in heme, nonheme and soluble forms in meat generally consumed in Thailand and influence of boiling, drying and freezing on the iron contents in these meat have not been reported. This study was

designed to

(1) generate data on the iron contents in the forms of heme, nonheme and soluble iron in beef, pork, chicken and other meat such as fish, mussel and shrimp;

(2) evaluate the influence of heat treatments, boiling and drying, and freezing, on the contents of the different forms of iron in these meat.