EVALUATION OF METHODS USED IN MEAT IRON ANALYSES AND
IRON CONTENT OF RAW, COOKED, AND CURED MEATS

by

Eldred Merlyn Clark

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1997
ABSTRACT

Evaluation of Methods Used in Meat Iron Analyses
and Iron Content of Raw, Cooked,
and Cured Meats

by

Eldred M. Clark, Doctor of Philosophy
Utah State University, 1997

This research project was divided into three parts. In the first part, heme, nonheme, and total iron methodologies for meats were evaluated. The accuracy, precision, and specificity of each method were determined by spike recoveries of heme and nonheme iron, and by analysis of National Institute of Science and Technology standard reference materials. The most reliable and practical methods were then used to determine the total, nonheme, and heme iron contents of various meats before and after cooking. The meats analyzed were beef, pork, lamb, chicken, and turkey. The wet-ashing technique was a novel procedure in which nitric acid was used to digest most of the solids followed by peroxy-monosulfuric acid to complete the digestion. Total iron values of the meats were consistent with those previously reported, but the percentage of heme iron in red meats was much greater than commonly assumed, both before and after cooking.
In the second part, the distribution of heme and total iron in heat-processed poultry products was investigated using light and dark chicken meat in the form of deep-fried chicken breasts and legs purchased from fast food restaurants and grocery stores in a ready-to-eat condition. Heme and total iron values were 1.7 ± 0.5 and 6.5 ± 2.0 µg Fe/g meat for light chicken meat and 7.6 ± 1.6 and 19.3 ± 2.2 µg Fe/g for dark chicken meat. Percent heme iron values averaged 29 and 40% for light and dark chicken meat, respectively.

In the third and final part, an application for the heme and nonheme iron data assembled above was developed to give dieticians an important tool in dietary formulations designed to maintain iron homeostasis. From the data it is evident that cooked light chicken meat, taken from the breast, would provide the lowest quantity of absorbable iron among the meats investigated and that ground beef, highest in heme iron, would provide the greatest quantity of bioavailable iron.

Additional research was performed on processed beef products. Cooked ground beef, frankfurters, beef steak, and roast beef were analyzed for heme and total iron. The different beef products contained similar amounts of total iron, 31.4 to 34.2 µg/g, but the heme iron content ranged from 6.2 µg/g in frankfurters to 36.3 µg/g in beef steak. Percent heme iron ranged from 33.0 to 63.8% in all meats. Total iron, heme iron, and percent heme iron varied significantly (P < 0.01) among meats, sources, and preparations. This research was published and has been reproduced in Appendix F.
ACKNOWLEDGMENTS

It is perhaps appropriate first to give thanks to God for life, liberty, intelligence, and a good inheritance, those things over which we individually have such little control. Such blessings are often overlooked in this secular, hedonistic world.

Second on the list of indebtedness is to Luella Clark, my mother, and to the late Dr. Arthur William Mahoney. I am indebted to my mother for unselfishly giving me life, for her dedication to her children, and for living an exemplary life. I am indebted to Dr. Mahoney for accepting me as a graduate student, for provided monetary support, for teaching scientific principles, for leading a noble life, and for being a constant friend. His loss has been felt by all who knew him. He not only directed and counseled with his students, but also became directly involved in their lives. He often brought them vegetables from his large garden, helped solve personal problems, and assisted in making needed car repairs. If any are deserving of the grace of God for service to his fellow man, Dr. Mahoney stands at the top of the list. He served well while here on the earth and will be remembered always by the many with whom he came in contact. May his knowledge, wisdom, and exemplary life live on in those who were fortunate enough to be influenced by him in some way.

Thanks is extended liberally to all members of the graduate committee, Drs. Hendricks, Cornforth, Aust, and Mendenhall, for their advice, time, and guidance during the course of this study and to Dr. Carpenter for taking over the position as chairman when Dr. Mahoney passed away.
The late Dr. Thomas Emery was also a great influence in guiding thought and direction of study the numerous times he was consulted, though he was too busy to serve on the graduate committee. His knowledge and wisdom, as revealed in his research and publications, will continue to improve the lives of us all.

Finally, the author expresses great gratitude to his wife, family, and mother-in-law: to Madeline, his wife, for going well beyond expectations in supporting and encouraging this great endeavor; to each family member (Chantelle, Shawn, Nicklaus, Kelly, Christopher, Ted, Meri, Cassandra, Daniel, Mac, Joshua, Joseph, Julianna, Rebekah, Skyler, and Jonathan) for their support, while their dad, a perpetual student, completed yet another degree; and to his mother-in-law, Goldie, for helping to support the family in untold ways. During this period of study, Madeline not only maintained a household for our 16 children, but also worked outside the home, continued her own educational pursuits that culminated in a second college degree, this one in nursing, made preparations for the marriage of two of our daughters, and gave birth to two of our wonderful children. The two daughters who married during this course of study, Chantelle and Meri, both had to finance the majority of their own marriages, because the family was partially dependent upon student loans for an income. The oldest daughter, Chantelle, after getting married to Tod Cox and establishing her own home, continued her support by giving gifts to replace furnishings, appliances, computer hardware, and clothing. May there be some benefit to all family members for the great sacrifices they all had to endure during these many years.

Eli Clark
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CHAPTER I
INTRODUCTION AND OBJECTIVES

INTRODUCTION

With few exceptions, all life forms have maintained a dependence upon iron for their existence. Most iron in our aerobic environment, however, is in an oxidized state, and very insoluble at neutral pH. Thus, with solubility normally being a prerequisite for availability, oxidized iron is usually unavailable to most living organisms. Because of this, most organisms have developed rather elaborate mechanisms to obtain their essential iron. Many plants and microorganisms, for example, produce siderophores, iron chelators, to bind exogenous iron for ultimate internalization. Some of these siderophores are so strong, they can extract iron from stainless steel or glass (Neilands, 1980). In the animal kingdom, carnivores and omnivores solve the problem of obtaining iron by consuming other animals rich in iron-containing proteins. Elsewhere in the ecosystem, the quest for iron is one of competition, most profoundly manifested in the virulent pathogenic microorganisms that parasitize other organisms, some of which are so competitive, they can extract iron from the host’s own iron-binding proteins (Weinberg, 1974).

Humans are no exception in their need for iron. In developed countries, much of this daily need comes from animal flesh. Meat, fish, and poultry are important constituents of the diet, accounting for as much as 80% of absorbed iron (Conrad et al., 1994).

The iron in meats is found in two different forms, as organic iron, most of which is bound in hemoglobin molecules, and as inorganic or nonheme iron. Each form is absorbed differently in the body. The absorption of nonheme iron is controlled according to
physiological need. For example, low body iron stores increase dietary nonheme iron absorption and high body iron stores reduce nonheme iron absorption. Heme iron, on the other hand, is bound in readily absorbable porphyrin ring structures, which are absorbed as much as 20 times more efficiently than nonheme iron.

Because of the body's ability to increase or decrease nonheme iron absorption depending upon physiological needs, nonheme iron absorption is rarely the cause of iron deficiency or excess. Heme iron absorption, however, because of the ease with which it is absorbed, readily contributes to iron overload.

The differential absorption of both heme and nonheme iron gives justification for knowing the different forms of iron in meats. This is discussed in the literature review of Chapter II. Methods of analysis for quantitatively determining the different forms of iron in meats, the primary objective of this research, are also reviewed in Chapter II.

To be able to determine the relative amounts of heme, nonheme, and total iron in each type of meat, accurate, precise, and specific methods of analysis must be developed for both fresh and heat-processed meats. These methods must then be universally tested and adopted as standard methods of analysis for universal applications. The results of research on heme and nonheme iron methodologies are recorded in Chapter III.

Following establishment of standard methods of analysis for heme, nonheme, and total iron; all the commonly consumed meats need to be analyzed and a database established for each meat type. The most commonly consumed beef and chicken preparations were collected from different sources over an extended time and analyzed for
each of the different iron fractions. Data accumulated from these meat preparations are reported in Chapter IV and in Appendix F.

Because iron is chemically very reactive, quantities in the body are limited, constantly bound in protein molecules, and closely controlled. Ideally, quantities of iron in the body are limited to functional need with some in storage to compensate for iron loss in bleeding. Since humans have a limited capacity to excrete excess body iron, iron balance is controlled by absorption rather than excretion, and absorption is optimally limited to physiological need. Heme iron absorption, however, readily exceeds the capacity of the body to regulate its uptake, and hence, total iron absorption is increased. Over time this will lead to excessive iron stores. High body iron stores are now known to increase the risk of a wide range of chronic health problems, including heart disease, cancer, brain dysfunction, endocrine abnormalities, and microbial infections.

To maintain iron homeostasis and to avoid both iron deficiency and iron excess, dietary recommendations cannot be based merely on the total iron content of meats. Total iron values are presently all that can be found in nutrient composition tables. Because of the differential absorption of both heme and nonheme, dietary recommendations must be based on the amount and availability of each type of iron in the diet.

To enable dieticians to estimate the potential bioavailable iron in an effort to maintain iron homeostasis, dietary heme and nonheme iron must be considered separately. From the data on heme and nonheme iron accumulated in this research, the bioavailability of iron in selected meats has been estimated and reported in Chapter V.
Conclusions on the most accurate, specific, and precise methods of analysis for heme, nonheme, and total iron are given in Chapter VI. Recommendations pertaining to the development of methodologies for meat iron determinations, the aim of future research in mineral analyses, and the potential use of accumulated heme and nonheme iron data can also be found in Chapter VI.

OBJECTIVES

The general objective of this study was to accumulate a database of heme and nonheme iron values from a variety of raw, cured, and heat processed meats. The specific objectives, which evolved over the course of the research, are listed below:

1. To establish accurate, precise, and specific methods of analysis for quantifying heme, nonheme, and total iron in both fresh, cured, and heat-processed meats.

2. To develop a database of heme and total iron values on a variety of beef products in a ready-to-eat condition.

3. To develop a database of heme and total iron values on various chicken products as prepared for consumption.

4. To calculate dietary data on potential iron availability from a variety of meats.

LITERATURE CITED


CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

This literature review chapter begins with a brief discussion of the justification for research in methodologies. It reveals the need for standardizing methods used in quantifying heme and nonheme iron in meat, fish, and poultry (MFP) analyses, and the need for developing a database of iron values for each of these meats. Justification for this research is grounded in the differential absorption of both heme and nonheme iron, and hence the first section of this review covers the topics of iron absorption, iron deficiency, and iron overload.

The second section reviews methodologies currently being used for the analyses of the different iron fractions in animal tissues. Analytical methods for quantifying heme, nonheme, and total iron are all covered in this section.

The final section of this literature review chapter concludes with reported variation in meat iron analyses with a perspective as to why these variations occur. Also included in this section are the effects of heat processing on heme and nonheme iron values. Most of the reported heme and nonheme iron values for meat products have been determined on raw meats. These values are not the same as those obtained from heat-processed meats, and hence are insufficient for determining the bioavailability of the various iron fractions in meats cooked for consumption.
JUSTIFICATION FOR RESEARCH

Physical health is characterized by a general homeostasis. Iron homeostasis can be defined as a dynamic state of iron equilibrium in which iron absorption equals iron loss, and implies that the amount of iron in the body remains constant. The assumption that physiological iron balance can be maintained by the amount of iron absorbed from a typical western diet is being challenged. For example, males after puberty and females after menopause reveal increasing iron stores (Cook et al., 1976), while new vegetarians (Heman and Darnton-Hill, 1987), athletes (Nickerson et al., 1989), and pregnant women (Halberg and Rossander-Hultén, 1991) are reported to be in a state of negative iron balance. Furthermore, it appears that the typical western diet, high in meat products, contributes to a chronic positive balance, which soon leads to iron overload (Leggett and Haliday, 1992). Hence, physiological control of iron absorption from a western diet is obviously not being accomplished. Research indicates that in western societies the primary consequence of a dietary iron imbalance is iron overload (Arthur and Isbister, 1987), a condition that greatly increases the risk of many life-threatening diseases.

Though blood transfusion and blood loss are certain causes of an iron imbalance, the primary cause of an imbalance in iron homeostasis is diet, and this results from the differential absorption of heme iron. Excess absorption of dietary inorganic iron, in association with alcohol, is another cause of excessive iron intake, as revealed by iron overload in the African Bantu (Derman et al., 1980), but this cause is minor compared to that of heme iron. Physiological adaption of the body to an increasing heme iron burden is incomplete and results in iron uptake greater than need (Bezwoda et al., 1983; Cook
Hence, understanding the mechanism and dietary factors that influence iron absorption is essential for maintaining iron homeostasis.

**Iron Absorption.** Regulation of body iron is accomplished through absorption rather than excretion. In 1937, McCance and Widdowson were able to show that the body has only a limited capacity to excrete iron. These findings have been confirmed over the years (Kreuzer and Kirchgessner, 1991) and the ability of the body to efficiently recycle iron has been made evident. Hence, except for blood loss, which subsequently reduces iron stores, body iron status is determined by iron absorption.

Absorption is defined as the passage of iron from the intestinal lumen, through the epithelial cells, and into the plasma for use in the body. Dietary iron taken up into the epithelial cells but not passed through the serosal membrane into the plasma for use in the body is merely exfoliated back into the intestine with the senescent epithelial cells and lost in the excreta.

Research has identified two pools of iron that function in absorption: heme and nonheme (Hallberg and Björn-Rasmussen, 1972). Each is absorbed differently in the intestine. Nonheme is absorbed as a cation whereas heme is absorbed as intact molecules (Halberg et al., 1979). Inside the mucosal cells, iron is split out of the porphyrin ring of the heme molecules by the enzyme, heme oxygenase (Raffin et al., 1974), and the iron then enters the same pool as the absorbed nonheme iron. The absorption of nonheme iron has been estimated to be as low as 2% for individuals with high iron stores and as high as 20% for those with low iron stores and consuming a meal rich in dietary iron absorption enhancers (Monsen and Balintfy, 1982). Enhancers such as ascorbic acid and meat
increase nonheme iron absorption but not heme iron absorption. Similarly, dietary inhibitors decrease nonheme iron absorption, but have little effect on heme iron (Carpenter and Mahoney, 1992). The most commonly encountered dietary inhibitors are found in grains, caffeinated beverages, and calcium containing foods.

In contrast to dietary nonheme iron absorption, 15-35% of heme iron has been reported to be absorbed from a typical western meal (Monsen, 1988). Lynch et al. (1989) reported that the absorption of heme iron does not appear to be affected by body iron stores and that people are susceptible to iron loading from high meat diets. This implies that regardless of the amount of iron already stored in the body, meat iron will be absorbed in excess of need. The differences in heme and nonheme iron absorption make it necessary that both iron fractions be known so that total iron absorption can be estimated and iron balance maintained.

Under normal physiological conditions, dietary iron absorption increases to match expanding body needs, as in growth or reproduction (Barrett et al., 1994). Low iron status markers during these times, however, have been largely misinterpreted and the subsequent diagnoses of iron deficiency inaccurately applied (Arthur and Isbister, 1987). A prime illustration of the misinterpretation of iron status markers is a report released by the U.S. Department of Health, Education, and Welfare. In the Ten State Nutrition Survey, 1968-1970 (1972), involving 30,000 people, a high percentage of iron deficiency was reported in men. Since blood loss is the primary way men become iron deficient (Crosby, 1974), how could such a high percentage of men be anemic? Other results were also questioned. For example, how could 95% of the women be eating iron deficient diets
with only 2% showing anemia (Crosby, 1979)? A follow-up random survey of 10,000 people, called the Health And Nutrition Examination Survey (HANES), similarly found more anemic men than women, 18% versus 11%, based solely on hematocrit measurements (United States Department of Health, Education, and Welfare, 1974). It has since been determined that iron deficiency cannot be diagnosed by looking at only one iron status marker (Cook et al., 1976) and that arbitrary limits for the iron status markers are not universally applicable. Researchers have also come to realize that iron status markers must be interpreted differently during growth and reproduction (Barrett et al., 1994; Koerper and Dallman, 1977; Yip et al., 1984).

**Iron Deficiency.** Most of the literature of the past (World Health Organization, 1959, 1968) and much of the more recent literature hypothesize that the primary cause of iron deficiency is poor iron absorption from predominantly vegetarian diets (Scrimshaw, 1991). Recent findings cast doubt on these long-held beliefs as illustrated by the affirmation of Arthur and Isbister (1987, p. 176): “It cannot be overstated that in adults the most cause of iron deficiency is blood loss and that nutritional deficiency as a sole cause must be exceedingly rare if ever a cause of iron deficiency.” Iron deficiency anemia is one of the most overdiagnosed diseases in the medical profession (Oppenheim, 1984), and some blood experts believe that it should be considered a “symptom” of occult blood loss rather than a “disease” (Elwood, 1977). Considerable evidence for this position has been published for each of the groups normally considered at risk of iron deficiency: 1) the aged, 2) gestating women, 3) infants, 4) children, and 5) vegetarians.
In the first population, the aged, it has been reported that more than 75% of the reported anemia in the elderly is a consequence of degenerative diseases (Johnson et al., 1994), which are not responsive to iron supplementation. These diseases give low iron status markers in spite of the high levels of stored iron that have accumulated since maturation in males and menopause in females. This is part of the “iron withholding” mechanism employed by the body in defense of the diseased condition. Indeed, iron supplementation would likely exacerbate the problem (Weinberg, 1984).

The second population considered at risk of iron deficiency, pregnant women, generally have iron supplementation routinely prescribed throughout the entire gestation period, but especially in the last trimester. Now, even this practice is being questioned (Barret et al., 1994; Hibbard, 1988). Calculations of iron needed for pregnancy are approximately 1000 mg (Baynes and Bothwell, 1990). This includes 230 mg for blood losses in birth (equivalent to one blood donation), 450 mg for the increase in red blood cell mass, 70 mg for the fetus, and about 70 mg for the afterbirth. With about half of this iron needed during the last trimester, at least 5 mg would need to be absorbed daily to meet these needs. Considering a nutrient density of 7 mg iron per 1000 Kcal of food in a typical American Diet (Windham et al., 1983), absorption would need to be about 64% with a daily diet of 2000 Kcal. Most scientific literature indicates that maximal dietary absorption is 3-4 mg/day in a state of iron deficiency anemia (Woods et al., 1990), and that iron supplementation is essential to meet the needs of pregnancy. Baynes and Bothwell (1990) stated quite emphatically that 5 to 6 mg of iron per day cannot be obtained from any diet and that iron supplementation would be needed. Recent research
findings indicate, however, that routine iron prescriptions are not needed during pregnancy and that iron supplementation may be harmful (Barrett et al., 1994). These conclusions were reached as a result of a study to determine whether the increased iron demands of pregnancy could be dietarily met by increased iron absorption. Absorption was measured at 12, 24, and 36 weeks of gestation with stable isotope iron-54 and iron-57. Respective iron absorption at each of the three stages of gestation was 7%, 36%, and 66%, indicating sufficient increase in absorption to meet the expanding demands of pregnancy. This is perhaps the first time it has been demonstrated that the body is capable of meeting the demands of pregnancy without intervention. There are times when iron supplementation during pregnancy is needed, but this must be the exception in a country like the United States where iron-rich foods are so abundant. Hibbard (1988) concluded that iron supplementation is valuable only with particular patients. The detrimental risks associated with iron supplementation to gravid women and infants, include prolonged pregnancy (Beischer et al., 1979), polycythemia (Emery, 1991), and increased susceptibility to infection (Weinberg, 1974). Hemminki and Rimpelä (1991) stated that routine iron supplementation for pregnancy may be more damaging than beneficial.

Another consideration seldom taken into account when estimating the iron needs of gravid women is the savings of iron by missed menstruation during pregnancy. The quantity saved would be approximately equivalent to that needed by the growing fetus (Emery, 1991). Furthermore, the increase in red blood cell mass, which results in a dilutional anemia, contracts back to normal following delivery, and there is no loss of iron.
Included in the third population routinely considered at risk of iron deficiency are infants. To mitigate this assumed threat, all baby formulas are supplemented with iron. Anemia may have been a major problem at the beginning of the 20th century (MacKay, 1928), but Burman (1972), as long as 25 years ago, showed that routine iron supplementation for infants was no longer justified in developed countries. McMillan et al. (1976) pointed out that infants are born with 75-80 mg of iron per kilogram of body weight and that this is about twice the concentration found in mature adults. Cavell and Widdowson (1964) reported that infants, shortly after birth, excrete 10 times more iron than they absorb, and serum ferritin levels are as much as 8 times higher than at one year of age (Leggett and Halliday, 1992). Before the new guidelines were published in 1989, the Recommended Dietary Allowance for infants from 0 to 6 months was 10 mg of iron (Food and Nutrition Board, 1980). It has since been lowered to 1 mg iron per kilogram body weight up to 6 months of age, after which it increases to 10 mg daily. Considering that human milk only contains 0.4 mg of iron per liter (Fransson and Lönnertal, 1984), and 0.3 mg after 4 months of lactation (Siimes et al., 1979), it would take at least 25 liters, or more than 27 quarts of breast milk daily, with 100% absorption, to satisfy these requirements. Even to satisfy the new requirements, a newborn infant weighing 4 kg would have to drink about 21 quarts daily and increase his intake to more than 50 quarts if he doubled his weight by 6 months, since absorption of breast milk iron has been reported as only 50% (McMillan et al., 1976; National Livestock and Meat Board, 1990).

The fourth population considered at risk of iron deficiency is growing children. Researchers have come to realize that this group presents a problem in the interpretation
of iron status indicators. Transferrin saturation, red cell protoporphyrin, and ferritin often show values in children that would indicate iron deficiency in adults (Milman and Cohn, 1984), but iron therapy has shown little or no improvement in iron status. Koerper and Dallman (1977) have suggested that these are physiological differences in the regulation of iron during growth, similar to the changes seen with inflammatory diseases, when iron withholding is evident, and hence, iron status indicators for children must be set at a lower level than for adults. When this is done, most of the assumed iron deficiency among American children is eliminated.

The final population considered at risk are vegetarians. Indeed, vegetarians and people who eat meat sparingly do not exhibit an abnormal iron status from low iron grains and vegetables (Craig, 1994; Freeland-Graves, 1988; Havala and Dwyer, 1988). Instead, they show increased resistance to heart disease (Burr and Butland, 1988; Fraser, 1988; Sullivan, 1983) and to other degenerative diseases which increase mortality (Chang-Claude and Frentzel-Beyme, 1993). The Tarahumara Indians of Mexico are prime examples. Their diet consists primarily of beans, corn, squash, and a little meat, yet they are known for their remarkable physical endurance. They reportedly can run 200 miles or more at high altitudes in competitive sports, lasting several days (Connor et al., 1978). This is also true of the Hunzas who live in the high Himalayas and who also thrive on a predominantly grain and vegetable diet. They are not only known for their endurance, but also for their youthful appearances and long lives, free of heart diseases, cancers, parasites, infections, malnutrition, and tooth decay (Taylor, 1964). Craig (1994), in summarizing his
article on the iron status of vegetarians, stated that the risk of iron deficiency among vegetarians was no different from that of nonvegetarians.

The incidence of iron deficiency throughout the world is frequently given as 30% of the population or about 1,300 million people (Skikne, 1988). Reported less frequently, however, is the incidence of parasitic infections that cause chronic blood losses. Hookworms plague over 900 million people, schistosoma (fluke worms) infect another 200 million, and malarial protozoa cause 200 to 300 million deaths every year (Scrimshaw, 1991). This amounts to over 30% of the world population. This gives epidemiological evidence for the comments of Arthur and Isbister (1987) that the major cause of iron deficiency is blood loss and not inadequate absorption of dietary iron.

In an attempt to prevent the assumed widespread risk of iron deficiency, iron fortification of refined wheat was mandated over 50 years ago in many western societies. Twenty years ago it was estimated that 25% of all absorbed iron in the United States was fortified iron. At that same time in Sweden, after 30 years of iron fortification, fortified iron accounted for 42% of absorbed iron (Crosby, 1978). More recently, in recognition of the decreased risk of iron deficiency in affluent societies and the availability of high iron diets, the advisability of iron fortification is being reconsidered (Olsson et al., 1995). Detrimental consequences of this practice, such as accelerated rates of iron accumulation (Crosby, 1978), and cellular oxidation associated with aging, coronary artery disease, carcinogenesis, stroke, and neurodegenerative and inflammatory disorders (Conrad et al., 1994), are being reported. MacPhail and Bothwell (1989) stated that iron fortification programs in developed countries were not established on sound principles and that there is
no good evidence that they have ever been effective. Crosby (1986, p. 15) stated the same thing more fervently: “The efficacy of adding iron to flour as a method of correcting iron deficiency has never been demonstrated.” Furthermore, the Delaney Amendment to the Federal Food, Drug, and Cosmetic Act of 1958 prohibits the addition of additives that induce cancer in man or animals when either ingested or tested (Schultz, 1981). One of the most prevalent diseases associated with iron overload is liver cancer (Gordeuk et al., 1994). Hence, the addition of iron to flour is illegal according to a strict interpretation of the Delaney clause, but the FDA continues to overlook this particular health risk.

Iron deficiency is a symptom of blood loss and not a disease (Elwood, 1977); therefore, iron fortification is beneficial to only a very small percentage of the population. Furthermore, iron fortification is now being determined to be detrimental to the majority of the population. Fortification masks the real causes of many diseases and according to Conrad and his associates (1994) should be discontinued. Fortification also poses a particular problem for roughly 14% of the people with hemochromatosis and contributes significantly to the iron burden of the rest of the population without this disease (Lauffer, 1991a). Denmark has already discontinued their mandatory iron fortification of flour (Milman et al., 1995).

The literature cited above indicate that iron deficiency is now relatively rare in western societies, even among the aged, gestating women, infants, children, and vegetarians, and that scientific concern, therefore, should be focused upon the potential dangers associated with diets high in absorbable iron, known to increase iron stores (Leggett et al., 1990). Such diets ultimately lead to a condition of iron overload.
Iron Overload. The term "iron overload" implies an excessive amount of iron accumulation in the body. Until fairly recently, the sole cause of iron overload was believed to be the genetic disease primary hemochromatosis (PH). This disease was thought to be quite rare and clinical signs of abnormal body functioning associated with overload were usually not evident until body iron stores were exceedingly high. The frequency of PH was given as 1 per 7000 hospital deaths or 1 per 20,000 hospitalizations (Edwards and Kushner, 1993). These estimates were based on autopsy studies and the appearance of clinical symptoms, which have since been recognized as poor estimators of the disease. Milder et al. (1980) stated that clinical symptoms of diabetes, arthritis, and impotence are often present for up to 30 years before hemochromatosis is diagnosed, and that many people die of secondary diseases associated with iron overload (e.g., cirrhosis, diabetes, and cardiomyopathy) before the primary cause of their illness is correctly, if ever, diagnosed. Clinical signs usually do not occur until the fourth or fifth decade of life, though some people accumulate iron so rapidly they die in their early twenties (Crosby, 1978).

Within the last decade numerous studies in many countries have shown that PH is not a "rare disease" but rather one of the most common genetic diseases known (Jonsson et al., 1991; Leggett et al., 1990; Milman et al., 1995; Olsson et al., 1995; Porto et al., 1992; Velati et al., 1990). In the United States the frequency of PH has been estimated to be 4.5 per 1000 among Caucasians of European extraction (Edwards et al., 1988). This would mean that about 14% or 1 in 7 would be heterozygous carriers. Though heterozygous carriers do not accumulate iron as rapidly as homozygotes, they still have
higher than normal iron stores and are at greater risk of iron-associated diseases (Stevens et al., 1988).

Until recently there has been little published research to indicate how much stored iron might be considered excessive amounts, amounts contributing to body dysfunction. It has now become evident that high iron stores, not associated with PH, are common in western societies (Lauffer, 1991a; Salonen et al., 1992; Stevens et al., 1994; Sullivan, 1989; Weinberg, 1990) and that the amounts, once considered normal, are greatly increasing the risk of those diseases seen in carriers of PH. Lauffer (1991a) has estimated that iron overload affects at least half of all men and postmenopausal women in the United States.

Aside from PH, the major cause of iron overload appears to be the consumption of heme iron as contained in meats (Leggett et al., 1990). Meats are responsible for up to 80% of dietary iron (Conrad et al., 1994), and heme iron absorption increases in proportion to the amount of heme in the diet (Bezwoda et al., 1983). Mechanisms that control the absorption of nonheme iron are ineffective in controlling the absorption of heme iron. All absorbed iron above physiological need goes into ferritin storage proteins.

As storage iron increases, the risk of iron-mediated free radical production increases proportionately. The iron needed for the genesis of free radicals is derived from a low-molecular-weight iron pool (Brittonet al., 1990; Jacobs, 1977b). Iron for this low-molecular-weight iron pool is supplied by the mobilization of iron from ferritin. Ascorbate is one of the many different biological reducing agents capable of mobilizing iron from ferritin (Reif, 1992). Free iron acts via the Haber-Weiss reaction to reduce the ubiquitous
peroxides that are released from normal cellular oxidation. By donating electrons to peroxide molecules, ferrous iron generates hydroxyl radicals (Aust et al., 1985). These highly reactive species then create chain reactions that continue damaging organic molecules until quenched by antioxidants. When the rate of damage exceeds the rate of repair, organ dysfunction results, and clinical symptoms of some disease are manifested.

At the cellular level the consequences of free radical attack involve all organs of the body and include mutations (Loeb et al., 1988), chromatid exchange, chromosome aberrations, the development of oncogenes, accelerated aging, premature cellular death (Cerutti, 1985), neutrophil dysfunction (Cantinieaux et al., 1987), scission of DNA strands (Toyokuni and Sagripanti, 1992), addition to double bonds (lipid peroxidation), abstraction of hydrogen from carbon atoms, and oxidation of sulfhydryl, thioester, and amino compounds (Loeb et al., 1988). Iron overload also suppresses the activities of the immune system by a reduction in phagocytosis, in migration of B and T lymphocytes, in the number of T helper cells, in the activity of natural killer cells, in the tumoricidal activity of macrophages (Weinberg, 1992), and in the repair of DNA lesions (Trenam et al., 1992). The clinical conditions resulting from this cellular damage include important and life-threatening processes such as cardiomyopathy (Lauffer, 1991b; Salonen et al., 1992; Sullivan, 1981, 1989), arthropathy (Blake et al., 1981; Gordon et al., 1974; Merry et al., 1991), neoplasia (Babbs, 1990; Hann et al., 1990; Knekt et al., 1994; Nelson et al., 1994; Selby and Friedman, 1988; Stevens et al., 1994), neurological dysfunction (Ben-Shachar and Youdim, 1993; Dexter, et al., 1989; Youdim et al., 1989, 1993), diabetes mellitus (Halliday and Powell, 1992), gonadal insufficiency or feminism (Gordeuk et al., 1994;
Jacobs, 1977a), and reperfusion injury (Babbs, 1985), in addition to increased risk of infection (Weinberg, 1974, 1984).

Because of the numerous oxidative processes occurring in the body, there is an elaborate system of self-defense to protect the body from free radical attack. The life span of man, compared to other mammals, is attributed to this superb free radical defense system (Cutler, 1984). The enzymes glutathione peroxidase and catalase are part of this protective scavenging system, and function by converting peroxide by-products into oxygen and water. Glutathione peroxidase will also remove lipid peroxides, and thereby stop the chain reaction of lipid peroxidation (Florence, 1991). Superoxide dismutase (SOD), another protective antioxidant enzyme, scavenges superoxide. Superoxide, like iron, is a powerful reducing agent, a nucleophile, or proton acceptor (Halliwell and Gutteridge, 1984), and must be controlled. There are a number of other nonenzymic molecules that function in the free radical defense system. These simple molecular antioxidants include ascorbic acid, reduced glutathione, alpha-tocopherol, beta-carotene, ceruloplasmin, apotransferrin, uric acid, albumin, and glucose (Halliwell and Gutteridge, 1984). Good health, therefore, free of radical-induced tissue damage, appears to be dependent upon the rate of radical production versus the rate of the scavenging system to dispose of these radicals. Anything that favors the production of free radicals, such as iron overload, or decreases the quantity of antioxidants would induce tissue damage by free radical production.

Because of the dangers associated with iron-catalyzed free radical production, the acquisition, transport, and storage of iron must be highly regulated. Indeed, the benefits
or detriments of iron will be determined by the ability of the body to control these functions, which ability is greatly impaired by high iron stores.

One of the first reports to show the consequences of high iron stores, not associated with PH, came from Finland. In this report it was shown that the risk of coronary heart disease was greatly increased proportional to the storage of iron in the body (Salonen et al., 1992). In the general population the risk of ischemic heart disease in men is four times greater than in women, until the age of menopause. At menopause the risk of heart disease increases to about the same as men (Kannel et al., 1976; Lauffer, 1991a). In 1981, Jerome L. Sullivan, hypothesized that the increased risk of heart disease between men and women was a result of higher iron stores in men. His hypothesis states that iron depletion protects against ischemic heart disease (IHD) and is based on the following observations: 1) Heart failure is prevalent in people with high iron stores; 2) IHD in men increases with age after adolescence, which parallels the increases in stored iron; 3) premenopausal women have a four-fold reduction in heart disease risk, which is lost at menopause, at which time iron stores begin to rise in proportion to the added risk of IHD.

Until fairly recently blood cholesterol level alone, and not iron, was implicated in the increased risk of IHD (Coronary Drug Project Research Group, 1973). The profound influence exerted by high cholesterol is evident by people with the genetic disease familial hypercholesterolemia (FH). Homozygotes of FH develop very high levels of serum cholesterol, six to eight times normal, and can have myocardial infarctions in childhood (Sullivan, 1989). Heterozygotes have cholesterol levels two to three times normal and do
not show clinical signs until the third or fourth decade of life. Since this inherited disease is autosomal, one would expect females and males to exhibit the same rate of IHD, but they do not. Until menopause, women with FH continue to be protected from IHD, in spite of their high levels of serum cholesterol. This suggests that there is more to IHD than just cholesterol level. Steinberg et al. (1989) reported that low-density lipoproteins (LDL) in the arterial walls must first be oxidatively modified to attract macrophages, which begin the atherosclerotic lesions that result in plaque buildup. Iron has been implicated as the mediator in this process (Parthasarathy et al., 1989).

The synergistic effects of iron and cholesterol have been visually characterized by Lauffer (1991a). To detect differences in the levels of cholesterol or in the incidence of heart disease between men and women, ratios are calculated. If there is no difference between the sexes, a ratio of one is obtained. When the male/female cholesterol ratio is plotted against age, the ratio at all ages is near one, and there is no variation to account for the increased risk of IHD in males. This has been illustrated in Figure 1. When the male/female heart disease rate is plotted against age, it shows approximately a four-fold difference between males and females during the reproductive years of 20 to 50. This four-fold difference is also the increased risk of IHD for males over females during these years. This can be seen in Figure 2. By plotting the male/female iron storage levels against age, this same four-fold difference is evident. This has been depicted in Figure 3. The plotting pattern produced is very similar to that evident in Figure 2, indicating that storage iron is indeed closely correlated with IHD, and that cholesterol alone is insufficient to explain the high levels of IHD in developed countries.
Figure 1. Male/Female Cholesterol Ratios (Lauffer, 1991a)

Figure 2. Male/Female Heart Disease Ratios (Lauffer, 1991a)
In spite of the recent numerous reports from different countries about the high frequency of hemochromatosis, the 1993 edition of Taber's Cyclopedic Medical Dictionary (Thomas, 1993, p. 874) still describes hemochromatosis as "a rare disease," and prominent medical doctors are still saying that hereditary haemochromatosis is not sufficiently prominent for it to be considered a public health problem (Hallberg, 1989). Furthermore, medical doctors are either ignorant of the consequences of high iron diets or are assuming that not enough is yet known to stimulate precaution among the populous, since they continue to prescribe iron without blood tests and recommend iron fortification of infant formulas based on an assumed need. Numerous reports indicate that infants have sufficient iron to meet their growth needs and that excess iron greatly increases the risk of numerous diseases and death (Barry and Reeve, 1977; Becroft et al., 1977; Brock, 1980; Emery, 1991; Haddock et al., 1991; Oppenheimer, 1989). False beliefs and fears about
the risks of iron deficiency at all stages of life have caused hemochromatosis to remain a rare disease (Niederau et al., 1985) and the hazards of excess iron accumulation to remain unknown in the minds of the public.

Death from diseases resulting from high body iron stores is tragic, because detection is easy and treatment can establish a normal life expectancy (Halliday, 1989). Detection must be done, however, before clinical signs of organ damage appear.

Treatment involves reducing iron stores by chelation therapy or repetitive phlebotomy. Chelation therapy is the treatment of choice for transfusion-dependent patients while phlebotomy is useful for everyone else. Each pint of blood removed carries with it about 225 mg of iron, slightly more for men and slightly less for women (Lauffer, 1991a).

**METHODS OF ANALYSIS**

There are currently no standard methods for measuring the iron content of raw or heat-processed meats, though several different methods have been developed. The Association of Official Analytical Chemists (AOAC) has yet to adopt a specific method for determining the heme, nonheme, or total iron content of meats (McNeal, 1990), and the USDA publication on the nutrient composition of foods, the Agriculture Handbook 8 series, gives only total iron values and does not differentiate between heme and nonheme iron when reporting meat iron content (Anderson et al., 1989, 1992; Anderson and Hoke, 1990; Posati, 1979).

Since heme and nonheme iron are absorbed differently in the intestines, a knowledge of both iron fractions is needed in order to be able to predict iron absorption,
to maintain homeostasis, and to formulate dietary recommendations designed to meet body needs. A general knowledge of heme and nonheme iron bioavailability has made it possible to develop a simple mathematical formula to predict iron uptake from the normal American diet (Monsen et al., 1978). This formula, however, uses a single value for the heme iron content of all meats (40%), and does not take into account the variable effects of heat processing on meat iron absorption. The percentage of heme iron in beef, for example, has been reported to be approximately 85% in ground beef (Oellingrath and Slinde, 1985) and less than 30% in beef franks (Kalpalathika et al., 1991). Furthermore, it is well established that heat alters the proportions of heme and nonheme iron in the meat product (Buchowski et al., 1988; Chen et al., 1984; Igene et al., 1979), and this greatly affects the bioavailability of the dietary iron.

Meat iron is divided into heme and nonheme sources because each has its own characteristics of absorption. Absorption of nonheme iron, for example, is greatly affected by both endogenous factors, e.g., body iron status (Magnusson et al., 1981), as well as exogenous factors, e.g., other dietary foods and chemicals (Fairweather-Tait, 1989; Hallberg, 1981; Morris, 1983), whereas heme iron absorption is only minimally affected by endogenous factors (Hallberg et al., 1979) and relatively unaffected by exogenous factors (Layrisse and Martinez-Torres, 1972). Because of these differences, heme iron is absorbed in much higher percentages than nonheme iron (Bezkorovainy, 1989; Conrad et al., 1966), and total iron measurements on foods are insufficient for determining dietary iron adequacy, especially when considering that meat in the diet can account directly or indirectly for up to 80% of absorbed iron (Conrad et al., 1994; Layrisse and
Martinez-Torres, 1972), though 50% is typical. It becomes necessary, therefore, in order to accurately estimate absorbable dietary iron contributed by meats to develop a database in which total iron is divided into heme and nonheme iron fractions. This must be done for all the commonly consumed meats in a ready-to-eat condition.

The value of knowing the proportions of each iron fraction in meats is emphasized when considering the consequences of negative or chronic positive iron balance. Negative iron balance, due to a monotonous low iron diet or chronic blood loss, leads to iron deficiency anemia, whereas positive iron balance due to continual ingestion of iron-dense foods, abnormal iron absorption, or repeated transfusions, leads to iron overload. Iron deficiency anemia or overload, though relatively long in developing, can impair the quality of life, or terminate it with an early death. Dietary selection becomes important, then, to assure iron balance and to avoid these detrimental consequences. Hence, with greater interest and importance being placed on iron balance as a prophylaxis for the numerous degenerative diseases possible as a consequence of imbalance, the need for a set of standard analytical methods for iron determinations in meats is evident. This begins with the acquisition of a representative sample of the meat under investigation, followed by a standard method of meat handling, sample preparation, mineral separation, and elemental analyses. The results of meat analyses can be no better than the analytical techniques upon which they are based.

**Meat Iron Analyses.** The first step in meat iron analyses is obtaining a representative sample of the meat to be analyzed. Considering the variations in mineral content that exist in the animal carcasses at different stages of maturity (Anderson and
Hoke, 1990), it is difficult to described a “representative” sample. In addition, meat cuts from different parts of the same animal yield different results (Kalpalathika et al., 1991). It becomes necessary, therefore, to analyze representative meat cuts from each animal separately (Schricker et al., 1982).

The next two steps in meat iron analyses are sample handling and sample preparation. Because of the low iron content of many meats, any contamination during sample handling would greatly distort the results, and there are numerous sources of environmental contamination (Narayanan and Lin, 1985; Thiers, 1957). Some of the greatest potential sources of contamination are the meat processor and holding vessels used in tissue disintegration (Pomeranz and Meloan, 1987). Stainless steel grinders or knives should be used, coupled with iron-free glassware cleaned in hydrochloric acid and rinsed in ethylenediamine tetraacetic acid.

The last two steps in meat iron analyses are mineral separation and elemental analysis. Mineral separation in meats is best accomplished by wet or dry ashing (Clegg et al., 1981a, 1981b). Since iron retention is a problem at temperatures that are too low or too high in dry ashing (Clark, 1976), preliminary experimentation is needed to obtain the best temperature for the tissue being analyzed. Residual carbon retains iron when temperatures are too low, and phosphates form a glassy mass that retains the iron at high temperatures (Peterson, 1952).

Developing and following a prescribed set of procedures for meat iron analysis leads to a method that is accurate, precise, and specific. Accuracy is the proximity of the replicate mean to the true value. Precision is a measure of the variation among replicates.
and trials. Specificity is the ability of the method to detect a measurable response due solely to the presence of the analyte. Spike recovery is a good measure of accuracy and specificity, both of which are strengthened by the use of certified standard reference materials (Wolf, 1982).

Quantitative measures of heme, nonheme, and total iron are dependent upon reliable methods of analyses. The following review reveals what has been developed for the determination of each of these iron fractions.

**Heme Iron Determinations.** Dietary heme was not important to nutritionists before 1950 because it was not believed to be absorbed by the human digestive system. Black and Powell (1942), however, published evidence to the contrary, but it was not until Walsh et al. (1955) in Australia and Callender et al. (1957) in Britain published the results of their findings using radioactive iron that heme was accepted as a dietary iron source. Since this time greater interest has been shown in finding a reliable method for the determination of heme iron in meat products.

In the past, various buffers, organic solvents, and even plain water have been used for heme pigment extraction (Warriss, 1979). Water did not appear to completely extract the myoglobin from muscle, buffer extracts were difficult to clear, and organic solvents gave variable results. Anderton and Locke (1955) were among the first researchers to publish a useful method. They were concerned, however, with the color of meat and not with iron content. They used acetone to wet the meat sample and extracted the heme pigments with ether. No estimate of the completeness of extraction was given. The following year Hornsey (1956) published what has become the standard with which to
compare all other heme iron methods. This method contains two different procedures: one for the extraction of nitric oxide-heme pigments and one for the extraction of all heme pigments, including hemoglobin, myoglobin, and the cytochromes. Total heme iron is calculated from the amount of acid hematin extracted. One early buffer extraction method was developed by Fleming et al. (1960). The typical problem of turbidity from buffer extraction methods was overcome by a combination of centrifugation and filtration. Warriss (1979) increased the pigment extraction efficiency of the buffer extraction methods by using 0.04 M phosphate buffer, pH 6.8. He then converting the extracted pigments to cyanmet compounds by adding potassium ferricyanide and sodium cyanide. Krzywicki (1982), by using the same extraction procedures of Warriss (1979), but not using the conversion step to cyanmet compounds, was able to quantify the various oxidative states of the heme compounds. This phosphate extraction method was reported to be as precise and accurate as the Hornsey method (Krzywicki, 1982), but because of the need for a clarification step involving high-speed centrifugation, this method is not as practical in terms of equipment and time.

Buchowski et al. (1988) reported that heme iron determined by the Hornsey method plus nonheme iron determined by a modification of the method of Schricker (Rhee and Ziprin, 1987) was approximately equal to total iron determined by wet ashing followed by colorimetric analysis using ferrozine. This provided some quantitative evidence for the specificity of the Hornsey method of analysis.

Though it is not important to know the fractions of hemoglobin and myoglobin for absorption measurements, since both are absorbed equally well, it becomes important
when meat quality is being considered. Myoglobin is reported to be more effective in catalyzing lipid oxidation (Silberstein and Lillard, 1978). Using high-performance liquid chromatography (HPLC), Oellingrath and Slinde (1985) were able to separate hemoglobin and myoglobin from beef extracts. Repeated use of the column, however, caused the hemoglobin and myoglobin peaks to run together, making the procedure impractical for general use. Additional research with different columns and a new technique of hydrophobic interaction enabled the researcher to ultimately report that the method was rapid, repeatable, and sensitive enough to use as a routine method of analysis for hemoglobin and myoglobin in meat samples (Oellingrath et al., 1990). Additional research by Han et al. (1994), using a size exclusion technique, produced an even more reliable method of separation with a lower standard deviation. They concluded that this technique was better than the cyanmet method of spectrophotometric heme iron determination, by possessing higher sensitivity and repeatability. No comparison was made with the Hornsey method of analysis.

There are several factors that affect pigment concentrations, but may not affect iron concentrations to the same degree. Since heme iron concentrations are calculated from the amount of pigment extracted, these factors should be considered in standardizing heme methodologies. Hornsey (1957) reported that light and oxygen reduced pigment concentrations in cooked cured meats, and stated that this was probably due to the dissociation of the nitroso-hemochrome complex. The effect of oxidation on total pigment concentration is unknown, and may be the source of some variability in reported heme iron values. Hence, standardization of sample handling procedures could eliminate
this potential problem. Wedzicha and Ladikos (1985, 1986) reported that phosphate buffers enhanced the ability of acetone to extract heme molecules from aqueous solutions. They noted also that some hematin is lost by adhesion to the filter paper and glassware, and gave an empirical equation to correct for these losses when the hematin chloride concentrations are 1.5% to 58%.

Though the hematin and cyanmet methods of meat pigment extraction have been demonstrated to be reliable and simple, variables such as pH, moisture content, and light must be standardized to obtain reproducible results. Fronticelli and Bucci (1963), for example, were unable to obtain sensible extraction of heme pigments at pH values greater than 6, and Wedzicha and Ladikos (1986) reported that extraction efficiency was dependent upon pH and hence appeared to be more important than the acetone content. Hornsey (1956), investigating the effects of various acetone:water ratios upon pigment extraction, found that the moisture in meat must be included in the 80:20 ratio of acetone:water. He emphasized the importance of accurately determining the moisture in meat samples to be analyzed, and recommended that heme analytical procedures, especially with cured meats, be done under low light conditions. See Appendix A.

**Nonheme Iron Determinations.** Brückman and Zondek (1940) reviewed earlier methodologies for nonheme iron determination. The extraction media used by these methods included one or more of the following compounds: water, hydrochloric acid (HCl), thioglycolic acid, sodium pyrophosphate, sodium hydrosulfite, hydroquinone, and trichloroacetic acid (TCA). The chromogen used was either thiocyanate, thioglycolic acid, or bipyridine. The authors compared the methods and reported the influence of time
and temperature on the extraction of nonheme iron. They noted that the quantity of nonheme iron extracted after 7 min of heating pyrophosphate was comparable to that extracted after 4 days of cold pyrophosphate. With the exception of turbidity in some of the extraction mixtures, many extraction mixtures looked promising. From these initial beginnings two distinct methods evolved: water extraction and acid extraction.

Sato and Hegarty (1971) used the water extraction method, followed by a nonheme iron chelation and a heme iron precipitation. Igene et al. (1979) continued with this extraction method, followed by an EDTA nonheme iron chelation, and TCA protein precipitation. Their results were similar to those of Sato and Hegarty (1971). Chen et al. (1984) used the same type of extraction but concluded with an acetone precipitation. Their results were again congruent with those published previously for water extracts. Regardless of the repeatability of this method of nonheme iron analysis, it was still criticized as underestimating the true nonheme values of meats (Rhee and Ziprin, 1987).

The improvement of an earlier acid extraction method using a trichloroacetic acid-pyrophosphate solution was undertaken by Foy et al. (1967). Varying the quantities of chemicals used and the heating time, they were able to eliminate the common turbidity found in this type of extraction solution and improve the method so that it could be used on specimens with low iron concentrations. Ummadi adapted this procedure for the use of ferrozine color reagent (see Appendix B).

Further modifications of the acid extraction method were provided by Torrence and Bothwell (1968). They used an HCl-TCA extraction mixture on human liver samples with good results. The samples were incubated at 65°C for 20 h. They validated the
method on samples with variable hemoglobin concentrations. Schricker et al. (1982) adapted the method of Torrance and Bothwell (1968) to the determination of nonheme iron in muscle meats. They increased the sample size, increased the concentration of the TCA extraction mixture, and increased the volume of the incubation mixture, while decreasing the volume of the color reagent. Rhee and Ziprin (1987), however, reported that the method of Schricker et al. (1982) probably overestimated the nonheme iron content of meats because of what they called a pigment effect. They modified the method to reduce the pigment effect by first adding sodium nitrite to the meat slurry before adding the acid extraction mixture. They reported significantly lower nonheme iron concentrations when sodium nitrite was added, in comparison with when it was left out. Chen et al. (1984) reported that nitrite prevented the release of heme iron by apparently stabilizing the porphyrin ring during the heated incubation period. They found no differences between heat-processed and raw samples that had first been treated with sodium nitrite. Buchowski et al. (1988) further modified this method to enable the use of the ferrozine color reagent in the final steps of this colorimetric analysis. The protocol for this method can be found in Appendix C. Ahn et al. (1993) added a clarification step to these extraction methods, by putting the samples in a refrigerator for 2 days following the incubation period. This produced a clear solution for the spectrophotometer.

Another nonheme iron method reported in the literature was a modification of the water extraction method of Carter (1971). This method was done by macerating a meat sample with mortar and pestle, followed by homogenization with a polytron in a citrate-phosphate buffer solution. The resulting homogenate was acidified, precipitated,
and centrifuged. The supernatant was mixed with a buffered color reagent and measured spectrophotometrically (Ahn et al., 1993).

**Total Iron Determinations.** The primary objective of a total iron analysis is the destruction of the organic matter by some form of digestion with the subsequent release of all the iron. There are methods of analysis that do not require the destruction of the organic matter, one of which will be discussed briefly below, but the equipment needed is costly and found in very few laboratories. The digestion method selected must prevent volatilization and adhesion losses, and minimize the production of insoluble compounds. The concentration of released iron can then be determined by mixing with a suitable chromogen, specific for iron (Appendix D), or measured by atomic absorption spectrophotometry (Appendix E). Meat extracts are unsuitable for total iron analysis, because they do not extract all the iron from the meat source, and hence, total iron values are lower than when analyzing intact lean muscle tissues (Chen et al., 1984).

The destruction of organic matter can be done either thermally (dry ashing), or chemically (wet ashing). Dry ashing is generally accompanied by significant iron losses (Clegg et al., 1981a; Friel and Nguyen, 1986), depending upon the heating conditions (Clark, 1976). In contrast, DeRuig (1986) reported no significant losses of minerals by dry ashing plant tissues. Hill et al. (1986), also found no significant losses of iron in a method using a combination of wet and dry ashing techniques. This method was claimed to reduce technician time required for analysis. Vuori et al. (1963) developed a method of wet ashing using the same vessel throughout the digestion process. This minimized the potential for positive contamination. Wet ashing, nevertheless, appears to be the
recommended method for ashing biological materials when quantifying iron (Carpenter and Clark, 1995; Clegg et al., 1981a; Friel and Nguyen, 1986; Kalpalathika et al., 1991).

Wet digestion techniques use many different oxidants to accomplish the digestion process. In a comparative study by Clegg et al. (1981b), nitric acid when used alone gave the best results in terms of percent recovery of total iron from certified liver samples obtained from the National Institute of Science and Technology (NIST). These researchers, however, recommended that nitric and perchloric acids be used in a ratio of 7 to 1 on samples high in fat.

A modification of the wet ashing technique has been reported in the literature (Oles and Graham, 1991). Instead of putting the acid digest on a hot plate for a day or two, it is put into a specially designed microwave oven. The digestion time is reduced to less than an hour for most organic tissues. It also has the advantage of eliminating the need for perchloric acid in high fat foods (cheese, nuts, potato chips, and chocolate), does not appear to be affected by incomplete digestion, and greatly reduces operator time. Results are comparable to hot plate digestions, even for highly volatile metals like zinc. Meats were also tested by this method of digestion with good results.

A nondestructive method of total mineral analysis was discussed by Nielson et al. (1988). This method uses X-ray fluorescence. It has the advantage of simultaneously providing multielemental analyses, is rapid, and seems to be more accurate and precise than atomic absorption spectrophotometry. The iron analyses were more variable than most other elements, but this is also common with dry ashing in preparation for AAS analyses (Clark, 1976).
In work by Davies et al. (1972), detection of iron in organic tissues by colorimetric and atomic absorption procedures following wet digestion gave means that were not significantly different. Variability, however, was greater for values obtained by atomic absorption than by colorimetric analyses. Ummadi (1991), using wet digests, found AAS values to be significantly lower than those by visible spectrophotometry. The differences could not be attributed to matrix interference. In contrast, Gordon (1978) reported that the ferrozine method was more variable than AAS in the analyses of sea foods, and also produced values that were about 8% lower.

There have been several chromogens developed from the ferroin group of compounds that act as bidentate ligands, capable of binding ferrous, cuprous, and cobaltous ions. The phenanthrolines and the pyridines, two chromogens from this group, have been used for iron determinations for many years with good success. Two new chromogens, however, ferrozine (Stookey, 1970) and ferene (Hennessy et al., 1984), have been reported to be able to detect lower iron concentrations and are lower in cost than the phenanthrolines or pyridines. Derman et al. (1989), in a comparative study of ferrozine, ferene, and bathophenanthroline disulphonic acid, concluded that both ferrozine and ferene should replace bathophenanthroline as the chromogen in serum iron measurements. The molar absorbance of the iron-ferrozine complex was 25% greater than that of bathophenanthroline, and iron-ferene complex was 50% greater. Increased absorption, as indicated by the higher extinction coefficients of both ferrozine and ferene, means greater sensitivity in terms of detection limits. This enables the detection of lower iron concentrations and the use of smaller sample sizes.
IRON CONTENT OF MEATS

There is little free iron in the body since most iron is bound by protein structures. Most of the functional iron is found in heme-containing proteins such as hemoglobin, myoglobin, and heme-containing enzymes. In humans this amounts to about two-thirds of the total. Humans, however, have more nonheme iron in storage molecules than animals. In a normal male this would amount to a third or more of total body iron (National Livestock and Meat Board, 1990). Animals have most of their surplus iron stored in the liver, spleen, and bone marrow (Chen et al., 1984). In slaughter, the blood is drained and the spleen and liver are removed. This effectively removes the major quantity of nonheme iron, since the transferrin is lost in the blood and most of the ferritin and hemosiderin are removed with the liver, spleen, and bones. Analyses of lean raw beef muscle indicate that there is 5 to 36% of the total iron in the nonheme form, depending upon the method of analysis (Igene et al., 1979; Rhee and Ziprin, 1987; Schricker et al., 1982).

Variations in Meat Iron Content. In addition to the variation in muscle iron content between animal species, there is widespread variability in reported heme, nonheme, and total iron values within species. For example, highly functional muscles, such as those in the legs of animals, would have more blood vessels, capillaries, and myoglobin and, hence, higher iron contents. Those muscles that are less active, such as those along the spinal column and ribs, or on the breast of chicken, would have lower iron levels. Other variables include incomplete bleeding during slaughter, the maturity of the animal, the degree of finish (feed lot fattening or grass fattening), the breed of the animal
(e.g., dairy breed or beef breed; broiler or fryer), the nutritional status, and the sex of the animal (Han et al., 1993).

Variations between laboratories, if not from variations within species, would probably be the result of different handling procedures and analytical methods. To minimize variations and to assure repeatability, all sample preparations, mineral separations, and elemental analyses should be standardized. The results of mineral iron analysis can be no better than the analytical techniques upon which they are based. Similarly, the sample selected for analysis must be representative of the species and tissue under investigation. These are elements of a quality control program, which should be part of any laboratory. All work performed should to be traceable to some reference standard, which has been certified by the proper agency, such as the National Institute of Standards and Technology.

Variability in the total iron analyses could be from the digestion process as well as from the instrument used. Dry ashing procedures will frequently give lower results than wet ashing (Clegg et al., 1981a; Friel and Nguyen, 1986), depending upon the temperature of the muffle furnace, the amount of time in the furnace, and the sample size (Clark, 1976). Though atomic absorption spectrophotometry (AAS) is as reliable and accurate as UV/VIS spectrophotometry procedures, there does seem to be a higher standard deviation with AAS analyses (Davies et al., 1972).

The two basic methods of nonheme iron analysis, water extraction and acid extraction, produce significantly different results. One is claimed to underestimate the true values and the other is claimed to overestimate them. For example, the results of the
modified Carter method compared well with other water extraction methods (Chen et al., 1984; Igene et al., 1979; Sato and Hegarty, 1971), but were lower than those extracted with acid (Rhee and Ziprin, 1987; Schricker et al., 1982). In comparing the two methods, Ahn et al. (1993) observed that the 20-h heated incubation period, in acidic conditions, probably was responsible for decreasing the heme iron and increasing the nonheme iron concentrations. They found no difference between the Schricker method (Schricker et al., 1982) and the modification by Rhee and Ziprin method (1987). Both methods employ the same acid extraction mixture and extended heating period.

**Processing Effects on Meat Iron.** Processing effects on meat iron have been investigated extensively and reported widely. Heat processing has a greater effect on iron than cold processing. Freezing temperatures do not appear to have much effect upon total, heme, or nonheme iron concentrations. There was a slight decrease in heme iron concentration over a 30-d storage period at -18°C, but it was not significant (Hamdaoui et al., 1992). Any type of heat processing, however, decreases heme iron and increases nonheme iron concentrations, depending upon the type, speed, duration, and severity of the treatment (Buchowski et al., 1988; Han et al., 1993), but has no effect upon the enhancement of nonheme iron absorption via the meat factor (Garcia et al., 1996). Heating also affects total iron concentrations by reducing the amount of moisture and thereby increasing the concentration of iron. Schricker and Miller (1983) observed that nonheme iron concentrations from a meat slurry increased linearly with cooking time. They reported that the normal cooking methods employed for meats, braising, broiling, and microwaving all have about the same effects upon heme and nonheme iron. They
observed about a 10% loss of measurable heme. Other heat processing methods are more severe, however, and have a greater effect upon heme iron content. Boiling beef for 90 min, for example, is reported to reduce heme iron content by 25% (Jansuittivechakul et al., 1985). Using rabbit meat, Garcia et al. (1996) observed an 87% loss of heme iron in the broth and a 22% loss in the solids. Hägerdal and Martens (1976) noted that heating at higher temperatures for a short period of time is equivalent to heating for a longer period of time at lower temperatures. Oellingrath (1988) reported a loss of 83.8% of the myoglobin and 70% of the hemoglobin when heated to 120°C for 1 h. Chen et al. (1984) reported that the rate of heating was more important to increasing nonheme iron than the final temperature. They reported a 5.34% increase in nonheme iron when heated to 70°C in 50 s, and a 71.75% increase when heated to 70°C in 8 min and 50 s.

Not only does heating reduce the heme iron content, but it also reduces the solubility of the iron. Garcia et al. (1996) reported a 50% reduction of soluble iron in addition to the 62% reduction in heme iron by boiling at “medium heat” until the meat was cooked. No specific temperature or criteria for doneness were given. Since heme iron content and iron solubility vary so widely with processing temperature, time, and conditions, heme and nonheme iron ratios cannot be known accurately without a complete iron analysis after heat processing. Considering the differential absorption of heme and nonheme iron from the intestines, it becomes obvious that the total availability of dietary iron is highly dependent upon heat processing and must be considered in dietary recommendations involving iron nutrition.
In the Hornsey method of heme iron analysis, heme iron is determined by measuring the total pigment concentrations in an acetone extract. Cooking has been reported to affect the pigment concentration (Buchowski et al., 1988; Schricker and Miller, 1983). Hornsey (1959) reported that total pigment concentration was higher for cooked pork than for raw pork by a factor of approximately 1.5. It is known that heat increases moisture loss and destroys the heme molecule (Schricker and Miller, 1983), but it is still unclear to what extent heat produces pigments that can be measured by the Hornsey method of analysis. Ummadi (1991) found a significant increase in heme values from well done meats. Evidently the cooking of meat to a well done condition increases the measurable pigment concentration, probably by the Maillard reaction of browning.

The effect of salts on the different iron fractions appears to be quite varied. Sodium chloride destabilizes the porphyrin ring of the heme molecule in meat during cooking and thereby increases the nonheme iron concentration (Ahn et al., 1993), whereas sodium nitrite stabilizes the porphyrin ring and prevents the loss of heme iron into the nonheme iron pool (Chen et al., 1984; Igene et al., 1979; Rhee and Ziprin, 1987).

Knowing the effects of heat on hemoglobin iron concentrates (HIC) is of value because this concentrate is being experimented with as an iron supplement in the food supply of developing countries to help combat the widespread iron deficiency that is prominent in these countries. Children are being given cookies or other foods enriched with HIC to help improve their iron status and to prevent iron deficiency anemia (Asenjo et al., 1985; Walter et al., 1993). The nonheme iron concentration of the HIC used was 20%, which did not increase with either fast or slow cooking. Heating to 150°C,
however, significantly increased nonheme iron (King et al., 1990). Though the degree of 
heme denaturation was not given, the authors concluded by stating that heating to 150°C 
or more would significantly decrease nutritional availability of the iron from HIC. 
Because of this they cautioned potential users to minimize heat processing when 
incorporating HIC into food products.

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CHAPTER III
EVALUATION OF METHODS USED IN MEAT IRON ANALYSES
AND IRON CONTENT OF RAW AND COOKED MEATS\textsuperscript{a,b}

ABSTRACT

The accuracy, specificity, and precision of several methods normally used to analyze iron in meats were assessed. The most reliable and practical methods were then used to determine the total, nonheme, and heme iron contents of various meats before and after cooking. Total iron was determined by using Ferrozine to detect the iron in wet-ash digests. The wet-ashing technique was a novel procedure in which nitric acid was used to digest most of the solids and peroxy-monosulfuric acid was used to complete the oxidation. Nonheme iron was determined by using Ferrozine to detect the iron in HCl-trichloroacetic acid extracts. Heme iron content was based on heme extracted into acidified acetone. Total iron values of the meats were consistent with those previously reported, but the percent of total iron present as heme in many meats was much greater than commonly assumed. This has important dietary implications since heme iron is the more bioavailable form of iron for humans.

INTRODUCTION

Iron is a trace element of considerable concern in public health. A complete, accurate, and quantitative knowledge of the levels and forms (heme or nonheme) of iron in

\textsuperscript{a} Reprinted with permission from the Journal of Agricultural and Food Chemistry, volume 43, pages 1824-1827, 1995, by the American Chemical Society.
\textsuperscript{b} Coauthored by Charles E. Carpenter and Eli Clark. See Appendix H.
foods is important since the bioavailability of each type of iron differs (Monsen et al., 1978). Meat is the main source of heme iron in human diets, and meat also makes a large contribution to the nonheme iron content of human diets (Carpenter and Mahoney, 1992). Levels of total, nonheme, and heme iron are often determined in meats, but little effort has been spent on validating the methods used to analyze iron.

Our first objective was to evaluate the accuracy, specificity, and precision of several methods now used to determine total, nonheme, and heme iron content in meats. We evaluated two total iron methods, two nonheme iron methods, and one heme iron method. The total iron methods used Ferrozine or atomic absorption spectrophotometry (AAS) to detect the iron in wet-ash digests. The wet-ashing technique was a novel procedure developed in our laboratory specifically for use with meat. It involved first digesting most of the solids with nitric acid and then employing peroxymonosulfuric acid, a strong oxidizer, to complete the oxidation. The nonheme iron methods used Ferrozine, a ferrous iron chromogen, to detect the iron in either HCl-trichloroacetic acid (TCA) extracts or sodium pyrophosphate-TCA extracts. The heme iron method was the acidified acetone extraction of Homsey (1956). Accuracy was determined by analysis of independent standards and by determining iron recovery from iron spikes added to ground beef (GB). The spikes were added to each lean, raw GB; fat, raw GB; or lean, cooked GB, to test if fat level or cooking interfered with the methods. Two measures of precision were calculated to reflect the repeatability (within-trial variability) and the reproducibility (between-trial variability) of each of the methods.
Our second objective was to determine the total, nonheme, and heme iron contents of various meats before and after cooking. The methods used were those found to be the most reliable and practical on the basis of our results from objective 1. The selected methods were total iron by Ferrozine detection of the iron in wet ash digests, nonheme iron by Ferrozine detection of the iron in HCl-TCA extracts, and heme iron by acidified acetone extraction. The meats and the cooking methods were as follows: ground beef, pan fry; beef round, braise; beef loin, broil; pork loin, broil; pork fresh picnic, roast; pork ham (cured), roast; lamb chop, broil; chicken breast, broil; chicken thigh, broil; turkey (ground), pan fry.

MATERIALS AND METHODS

Preparation of Ground Beef Samples. Lean GB was prepared by first trimming round roasts of all visible fat and grinding twice through a 1/8-in. plate. Fat GB was prepared by adding trimmed fat back into the lean GB as it was reground through the 1/8-in. plate. Cooked, lean GB was prepared by immersing hermetically sealed polyethylene bags of lean GB into boiling water until an internal temperature of 71°C was obtained. All samples were bagged in 3.00-mil polyethylene bags [0.75 mil of nylon laminated with 2.25 mil of 6% ethylene vinyl acetate and 94% polyethylene (Koch, Inc., Kansas City, MO)], vacuum sealed at 29-30 in. of Hg in a Vacu-fresh vacuum chamber machine (Meat Packers and Butchers Supply Co., Los Angeles, CA), and stored frozen at -18°C until used. Fat was determined by ether extraction (AOAC, 1990) using a Goldfisch fat extractor from Labconco (Kansas City, MO). The percent fat (mean ± SD) was 3.6 ± 0.6 in lean GB, 14.8 ± 3.0 in fat GB, and 4.6 ± 0.4 in cooked, lean GB.
Procedures. **Total Iron Methods.** Meat samples (ca. 2 g) were accurately weighed into 125-mL Erlenmeyer flasks, and 15 mL of concentrated nitric acid was added. Each flask was left to predigest at room temperature for 4-6 h or overnight. The flasks were placed on a hot plate set at 100°C until dry. Hydrogen peroxide-sulfuric acid reagent (Hatch et al., 1985) containing peroxymonosulfuric acid was added in 1-mL aliquots to each sample until they all became clear, typically after three or four additions. The flasks were left on the hot plate until all peroxide was expelled (5-10 min) and the white vapors of sulfuric acid became evident. The clear digest was allowed to cool and quantitatively transferred to 10-mL volumetric flasks using 0.01 N HCl as the rinse. Aliquots of the digests were analyzed for iron using Ferrozine color reagent or AAS. The Ferrozine method was as described by Carter (1971) and Stookey (1970), except that the final mix was 1 mL of sample, 1 mL of 1% ascorbic acid, 1 mL of 20% ammonium acetate, 1 mL of 1mM Ferrozine, and 1 mL of water. For AAS, standards were prepared in a reagent blank solution that had received the same wet ashing treatment as the samples, including equal additions of the peroxymonosulfuric acid reagent.

**Nonheme Iron Methods.** Meat samples (ca. 2 g) were weighed into Teflon-sealed screw-cap culture tubes. An extraction solution plus 0.1 mL of 1% sodium nitrite was added to the tubes. The extraction solution was either 15 mL of a 1:1 mixture of 40% TCA/6 N HCl (Schricker et al., 1982; Torrence and Bothwell, 1968) or 10 mL of a 7:3 mixture of 20% TCA/saturated sodium pyrophosphate (Foy et al., 1967). The mixtures were homogenized for 15 s with a kinematica polytron (Luzern, Switzerland), and the tubes were sealed and placed in a hot water bath for 18 h. After cooling, the mixtures
were centrifuged at 2000 g for 10 min, and the supernatants were filtered (GF/A filter paper, Whatman, Maidstone, England). The iron concentrations of the filtrates were determined with Ferrozine.

*Heme Iron Method.* Hemin was determined using the acidified acetone extraction of Hornsey (1956), with slight modifications. A sample of meat (ca. 10 g) was placed in a 50-mL centrifuge tube, and 20 mL of acetone and 0.5 mL of HCl were added. Water was added so that total water in the tube, both from the meat and from the added water, equaled 4.5 g. The mixture was processed for 15 s with a Kinematica polytron and filtered. The absorbance of the filtrate at 640 nm was measured, and heme iron in the sample was calculated. Water content of the meat samples was determined by drying at 105°C for 16 h (McNeal, 1990).

**Experimental Design.** *Evaluation of Iron Methods.* We tested the ability of the total iron methods to recover iron in various standards, including National Institute of Science and Technology (NIST) certified standards of wheat flour (SRM 1567), bovine liver (SRM 1577a), and hemoglobin. Four trials using triplicate samples were performed. Further, we tested the ability of all the iron methods to recover spikes of 20 μg of ferric iron or 13.4 μg of heme iron added to 2-g samples of lean GB, fat GB, and cooked, lean GB. The ferric iron spikes were 1-mL aliquots of 20 mg of ferric iron/L in 0.1 N HCl. The heme iron spikes were 1-mL aliquots of 40 g of hemoglobin/L, prepared by dissolving lyophilized bovine hemoglobin (Sigma Chemical Co., St. Louis, MO) in water. The iron content of the heme iron spike was calculated using the factor of 3.35 mg of iron/g of hemoglobin (Mahoney et al., 1974). Controls had 1 mL of double-demineralized water in
place of the iron spike. Five trials using triplicate samples were performed, and percent recovery of the spikes was reported as 95% confidence intervals.

*Iron Content of Raw and Cooked Meats.* The following methods were used to determine the iron content of various retail meats before and after cooking: total iron by Ferrozine detection of iron in wet ash digests, nonheme iron by Ferrozine detection of iron in HCl-TCA extracts, and heme iron by the Hornsey methodology. The retail meat samples (10) were purchased at five different retail outlets on two separate days. The meats and the cooking methods were as follows: ground beef (lean), pan fry; beef round, braise; beef loin, broil; pork loin, broil; pork fresh picnic, roast; pork ham (cured), roast; lamb chop, broil; chicken breast, broil; chicken thigh, broil; turkey, (ground), pan fry. All meats were cooked to 71°C internal temperature. ANOVA was employed to determine the effects of cooking and meat type on iron content.

**RESULTS AND DISCUSSION**

**Evaluation of Iron Methods.** *Total Iron Methods.* The total iron values determined with Ferrozine or AAS were not different from the certified values for NIST standards and were similar to the calculated value of 3.35-3.38 mg of Fe/g of hemoglobin (Table 1). Thus, these total iron techniques performed well on standard reference materials and showed promise for use with meat. Preliminary experiments on meat indicated our procedure had several advantages compared to other techniques (Clegg et al., 1981a,b; Friel and Nguyen, 1986; Hill et al., 1986; Kalpalathika et al., 1991; Schricker et al., 1982), including complete digestion that required no filtering of fat or other undigested components, speed of digestion, and no requirements for special equipment,
Table 1. Total Iron in Various Reference Materials and Ground Beef

<table>
<thead>
<tr>
<th>ref material*</th>
<th>total iron (µg/g)</th>
<th>ferrozineb</th>
<th>AASb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat Flour (SRM 1567)</td>
<td>14.1 ± 0.5</td>
<td>13.3 ± 0.5</td>
<td>14.0 ± 0.9</td>
</tr>
<tr>
<td>Bovine Liver (SRM 1577a)</td>
<td>194 ± 20</td>
<td>197 ± 4</td>
<td>196 ± 9</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>3350</td>
<td>3220 ± 50</td>
<td>3280 ± 80</td>
</tr>
<tr>
<td>Lean GB</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td></td>
</tr>
<tr>
<td>Fatty GB</td>
<td>23 ± 1</td>
<td>23 ± 1</td>
<td></td>
</tr>
<tr>
<td>Lean, Cooked GB</td>
<td>25 ± 1</td>
<td>24 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

* Reference values for liver and wheat are NIST-certified values. Reference value for bovine hemoglobin is as calculated by Mahoney et al., 1974. b Ferrozine and AAS values are means ± 95% confidence limits based on four trials using triplicated samples (standards) or five trials using triplicate samples (ground beef).

such as perchloric acid hoods. Gordon (1978) reported that Ferrozine gives 8% lower values than AAS after wet digestion of seafood using HNO₃-HClO₄. However, in our hands, Ferrozine and AAS gave similar values for total iron in the standards and in GB (Table 1). The lower detection limit of the Ferrozine assay is 0.090 µg of iron (Carter, 1971), which, for our digestion, translates into about 0.5 µg of iron/g of meat.

Total iron methods recovered all of the iron added as FeCl₃ spikes, but only 93-95% of the iron was recovered from Hb spikes (Table 2). Recovery was especially low from fat GB or cooked GB, indicating possible problems in these samples. Since the iron values determined using Ferrozine or AAS detection were similar, the low recovery of heme iron was probably due to incomplete digestion. Fat is notoriously hard to digest, and cooking may form compounds that are also hard to digest. The repeatability and reproducibility using Ferrozine or AAS iron detection were similar (Table 3). However,
Table 2. Recovery of Iron from Ferric Chloride and Hemoglobin Spikes Added to Lean Ground Beef, Fat Ground Beef, and Cooked, Lean Ground Beef

<table>
<thead>
<tr>
<th>spike and meat</th>
<th>percent recovery</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total iron</td>
<td>nonheme iron</td>
<td>heme iron</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>ferrozine</td>
<td>AAS</td>
<td>HCl-TCA</td>
<td>Na$_4$P$_2$O$_7$</td>
</tr>
<tr>
<td>FeCl$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lean GB</td>
<td>99 ± 10</td>
<td>100 ± 7</td>
<td>105 ± 8</td>
<td>110 ± 10</td>
<td>-11 ± 12</td>
</tr>
<tr>
<td>Fatty GB</td>
<td>100 ± 6</td>
<td>98 ± 6</td>
<td>107 ± 8</td>
<td>108 ± 10</td>
<td>-5 ± 7</td>
</tr>
<tr>
<td>Cooked GB</td>
<td>98 ± 10</td>
<td>100 ± 9</td>
<td>106 ± 7</td>
<td>109 ± 8</td>
<td>2 ± 10</td>
</tr>
<tr>
<td>All Meats</td>
<td>99 ± 4</td>
<td>99 ± 3</td>
<td>106 ± 3</td>
<td>109 ± 8</td>
<td>-5 ± 5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lean GB</td>
<td>98 ± 5</td>
<td>96 ± 5</td>
<td>11 ± 7</td>
<td>11 ± 4</td>
<td>96 ± 11</td>
</tr>
<tr>
<td>Fatty GB</td>
<td>94 ± 4</td>
<td>92 ± 3</td>
<td>11 ± 11</td>
<td>11 ± 4</td>
<td>103 ± 9</td>
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<tr>
<td>Cooked GB</td>
<td>93 ± 7</td>
<td>92 ± 6</td>
<td>11 ± 11</td>
<td>14 ± 5</td>
<td>105 ± 5</td>
</tr>
<tr>
<td>All Meats</td>
<td>95 ± 3</td>
<td>93 ± 2</td>
<td>11 ± 4</td>
<td>12 ± 2</td>
<td>101 ± 4</td>
</tr>
</tbody>
</table>

*Values are means ± 95% confidence limits for percent recovery based upon five trials using triplicate samples.

Table 3. Precision of the Iron Methods

<table>
<thead>
<tr>
<th>method</th>
<th>RSD (%) for repeatability</th>
<th>RSD (%) for reproducibility</th>
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</thead>
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<tr>
<td>Total Iron</td>
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</tr>
<tr>
<td>Ferrozine</td>
<td>3.8</td>
<td>2.5</td>
</tr>
<tr>
<td>AAS</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Nonheme Iron</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCl-TCA</td>
<td>8.0</td>
<td>22</td>
</tr>
<tr>
<td>Pyrophosphate</td>
<td>5.7</td>
<td>14</td>
</tr>
<tr>
<td>Heme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hornsey</td>
<td>2.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

-
these experiments were performed by personnel with significant experience with AAS. It was our experience that the Ferrozine method is a more rugged method than AAS and less susceptible to variability from operator error and matrix effects. Additionally, the Ferrozine method is quickly adaptable to any laboratory with a spectrophotometer.

**Nonheme Iron Methods.** The nonheme methods recovered 106-109% of the iron in FeCl₃ spikes and recovered 11-12% of the iron from Hb spikes (Table 2). The reason for the greater than 100% recovery of nonheme iron is unclear, whereas the recovery of some heme iron indicated that a substantial amount of heme was degraded into porphyrin and ionic iron during the extraction procedures (Ahn et al., 1993). Among the iron methods tested, the nonheme methods showed the most variability, with the HCl-TCA procedure somewhat more variable than the pyrophosphate-TCA extraction (Table 3). The HCl-TCA and sodium pyrophosphate-TCA extraction methods are routinely used for nonheme iron analysis in food samples, and both methods have the advantages of being simple and requiring low operator time per sample. The major drawback of both the methods is that they are not rapid techniques. The HCl-TCA extraction was preferred because of its consistently more accurate recovery of nonheme iron.

**Heme Iron Method.** The Hornsey method accurately recovered the heme iron spikes (Table 2). It was also the most repeatable and most reproducible of the iron methods (Table 3). The Hornsey method is one of the most commonly used methods for quantifying heme iron in food samples. It is relatively safe, fairly simple, and rapid. Also, the sources of contamination are minimal, allowing for accurate analysis of low concentrations of heme iron.
Iron Content of Raw and Cooked Meats. The following methods were used to determine the iron content of various meats before and after cooking: total iron by Ferrozine following wet ashing of the samples using nitric acid and peroxymonosulfuric acid, nonheme iron by Ferrozine after HCl-TCA extraction of the samples, and heme iron by the Hornsey methodology. The different meats varied \( p < 0.01 \) in their content of total, nonheme, and heme iron (Table 4), and iron values were generally consistent with

<table>
<thead>
<tr>
<th></th>
<th>total iron (( \mu g/g ))</th>
<th>nonheme iron (( \mu g/g ))</th>
<th>heme iron (( \mu g/g ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>raw</td>
<td>cooked</td>
<td>raw</td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground</td>
<td>22±5</td>
<td>26±3</td>
<td>5.5±1.8</td>
</tr>
<tr>
<td>round</td>
<td>22±2</td>
<td>37±4*</td>
<td>5.1±1.6</td>
</tr>
<tr>
<td>loin</td>
<td>25±4</td>
<td>34±4*</td>
<td>7.1±0.9</td>
</tr>
<tr>
<td>Fresh Pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground</td>
<td>7.1±1.3</td>
<td>10±2*</td>
<td>4.3±0.8</td>
</tr>
<tr>
<td>round</td>
<td>12±2</td>
<td>14±2</td>
<td>4.2±0.8</td>
</tr>
<tr>
<td>Cured Pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ham</td>
<td>7.3±1.3</td>
<td>8.4±1.2</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>Lamb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chop</td>
<td>16±1</td>
<td>17±1</td>
<td>5.2±1.2</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>4.3±0.4</td>
<td>5.4±0.4*</td>
<td>2.9±0.4</td>
</tr>
<tr>
<td>thigh</td>
<td>8.5±0.9</td>
<td>11±1.0*</td>
<td>4.7±1.0</td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground</td>
<td>13±1</td>
<td>16±1.0*</td>
<td>7.4±2.0</td>
</tr>
<tr>
<td>LSDb</td>
<td>2.6</td>
<td>3.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* The reported values are mean ± SD for \( n = 10 \) samples. The samples were purchased at five different retail outlets on two separate days. \(^b^\) Fisher’s least significant difference test for the column when \( F \) was significant at \( p < 0.01 \). * An asterisk signifies a significant difference between iron content of raw and cooked using Student’s \( t \) test at \( p < 0.01 \).
those we have previously reported (Buchowski et al., 1988; Carpenter and Mahoney, 1992). The total iron values did not equal the sum of nonheme iron and heme iron, probably due to a combination of overestimation of nonheme iron and underestimation of total iron (particularly its heme iron fraction) as previously discussed. The percent of heme iron to total iron varied among meats and was decreased by cooking (Table 5). The loss of heme iron with cooking has been attributed to the breakdown of heme into ionic iron and porphyrin (Ahn et al., 1993; Buchowski et al., 1988).

Table 5. Heme Iron as a Percent of Total Iron in Cooked Meats

<table>
<thead>
<tr>
<th>meat</th>
<th>percent heme iron</th>
<th>raw</th>
<th>cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground</td>
<td>91 ± 9</td>
<td>79 ± 9</td>
<td></td>
</tr>
<tr>
<td>round</td>
<td>99 ± 5</td>
<td>55 ± 6*</td>
<td></td>
</tr>
<tr>
<td>loin</td>
<td>90 ± 5</td>
<td>75 ± 9*</td>
<td></td>
</tr>
<tr>
<td>Fresh Pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>loin</td>
<td>69 ± 5</td>
<td>22 ± 6*</td>
<td></td>
</tr>
<tr>
<td>picnic</td>
<td>81 ± 4</td>
<td>65 ± 10*</td>
<td></td>
</tr>
<tr>
<td>Cured Pork</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ham</td>
<td>83 ± 5</td>
<td>79 ± 6</td>
<td></td>
</tr>
<tr>
<td>Lamb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chop</td>
<td>92 ± 3</td>
<td>80 ± 5*</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>42 ± 5</td>
<td>25 ± 4*</td>
<td></td>
</tr>
<tr>
<td>thigh</td>
<td>60 ± 3</td>
<td>32 ± 6*</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ground</td>
<td>70 ± 15</td>
<td>40 ± 8*</td>
<td></td>
</tr>
<tr>
<td>LSDb</td>
<td></td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

a The reported values are means ± SD for n = 10 samples. The samples were purchased at five different retail outlets on two separate days. b Fisher’s least significant difference test for the column when F was significant at p < 0.01. * An asterisk signifies a significant difference between % heme iron of raw and cooked using Student’s t test at p < 0.01.
Significance. Reliable data are needed concerning the heme and nonheme iron content of meat. This study has established the reliability of several methods presently used to determine total, nonheme, and heme iron in meat and then used the most reliable methods to measure the iron contents of a variety of fresh and cooked meats. This has important dietary implications since heme iron is the more bioavailable iron form for humans. The Monsen model (Monsen et al., 1978) is the most commonly used model for predicting iron bioavailability in individual meals. The Monsen model uses the value of 40% for the percent heme iron to total iron in meat, fish, and poultry (MFP). However, the meats examined here contained widely differing amounts and percentages of heme iron. This suggests that the value used in the Monsen equation should not be an unvarying 40% but should be different for each particular meat consumed with a meal. The Monsen model has been modified for use with populations (Carpenter and Mahoney, 1992). For use with this model, the average percent iron found as heme in MFP was estimated as 45%. This still appears to be a reasonable estimate based on the general pattern of MFP consumption in the United States (National Live Stock and Meat Board, 1994) and our data on the content of heme iron in various meats. However, this value may vary considerably depending on the consumption pattern of MFP for a specific population. On the basis of the meats examined here, the percent MFP iron found as heme will be greater than 45% for people consuming a large proportion of their MFP as red meats but will be less than 45% for people consuming a large proportion of their MFP as fish and poultry.
LITERATURE CITED


CHAPTER IV
HEME AND TOTAL IRON IN READY-TO-EAT CHICKEN

ABSTRACT

Distribution of heme and total iron in heat-processed poultry products was investigated with light and dark chicken meat in the form of deep-fried chicken breasts and legs purchased from fast-food restaurants and grocery stores. Heme iron content was determined by the Hornsey method, and total iron was determined by the use of Ferrozine on a wet-ashed digest. The heme and total iron were, respectively, \( 1.7 \pm 0.5 \) and \( 6.5 \pm 2.0 \) \( \mu g \, Fe/g \) meat (mean \( \pm \) SD) for light chicken meat and \( 7.6 \pm 1.6 \) and \( 19.3 \pm 2.2 \) \( \mu g /g \) for dark chicken meat. Heme iron values averaged 29 and 40% for light and dark chicken meat, respectively.

INTRODUCTION

There are two types of dietary iron based on different mechanisms of absorption: nonheme and heme. Nonheme iron, found in plant and animal products, has a low bioavailability ranging from 2-20% (Monsen and Balintfy, 1982), and is influenced greatly by a variety of enhancing and inhibiting components in the diet (Bothwell et al., 1989; Carpenter and Mahoney, 1992; Monsen, 1988). Heme iron, on the other hand, is found only in meat, fish, and poultry (MFP), has a much higher bioavailability, ranging from

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\(^{b}\) Coauthored by Eli M. Clark, Arthur W. Mahoney (deceased), and Charles E. Carpenter.
15-35% (Monsen and Balintfy, 1982), and is not affected by other dietary constituents. Because of these great differences in bioavailability between nonheme and heme iron, the relative quantities of dietary nonheme and heme must be known to accurately estimate the total amount of bioavailable iron in a food.

Most literature on the mineral composition of foods contains only total iron analyses with no breakdown into the heme and nonheme iron fractions (Duewer et al., 1993; McCance and Holland, 1991). However, heme iron percentages in raw MFP vary from 42% in chicken breast meat to > 90% in red beef (Carpenter and Clark, 1995; Chen et al., 1984). Furthermore, cooking degrades the highly available heme iron into the less-available nonheme iron (Buchowski et al., 1988; Carpenter and Clark, 1995; Han et al., 1993; Jansuittivechakul et al., 1986; Schricker and Miller, 1983). These findings emphasize the need to consider heme and nonheme iron concentrations in the meat products as normally consumed. Research on ready-to-eat beef products has already been reported (Kalpalathika et al., 1991). In this research, we determined the heme and nonheme iron concentrations of heat-processed, ready-to-eat chicken products.

MATERIALS AND METHODS

Sample Preparation. All meat samples were purchased from fast-food restaurants or grocery store delicatessens as whole meat products in a ready-to-eat condition. Both light (breast) and dark (drumsticks) chicken meats were collected at four different times from six different locations for a total of 24 samples of each type of meat. Samples were deboned, where necessary, trimmed of all visible fat and connective tissue, chopped finely with a stainless steel knife, put into aluminum pouches to minimize light
exposure and moisture loss, and weighed into vessels for moisture, heme, and total iron determinations. Moisture and heme iron analyses were performed on the day of meat purchase, and total iron analysis was started.

**Chemical Analyses.** *Total iron.* Values for total iron were obtained by wet ashing triplicate samples (0.5-1g) of both light and dark chicken meat. Standard reference materials (SRM) were purchased from the National Institute of Science and Technology (NIST), including bovine liver (SRM 1577a) and wheat flour (SRM 1567). These NIST samples were ashed separately at the same time as were the chicken samples. Samples and NIST standards were weighed into 25-mL Erlenmeyer flasks and ashed, first with concentrated nitric acid and then with 30% hydrogen peroxide at nonboiling temperatures, until a white ash was obtained. In recognition of the toxic fumes and possible explosion hazard, all digestions were performed in a perchloric acid hood, and safely glasses were worn at all times.

The resulting white ash from samples and standards was dissolved in 1 mL of 0.5 N HCl and transferred into 13 x 100-mm test tubes. Standards were prepared by adding 1-mL aliquots of FeCl₃ (1, 2, 4, 8, and 10 µg Fe/mL) diluted from a 1000-µg Fe/mL stock solution (Fisher Scientific, Pittsburgh, PA). One milliliter of freshly prepared 1% (w/v) ascorbic acid in 0.2 N HCl was added to each test tube and mixed. After 15 min, 1 mL of 20% ammonium acetate and 1 mL of 1 mM ferrozine were added, and the volume was made to 5 mL with deionized water. The mixture was allowed to stand in the dark for 45 min, and the absorbance was measured at 562 nm against a reagent blank with a UV-2100U recording spectrophotometer (Shimatzu, Columbia, MD).
**Heme iron.** Heme iron values were determined by the Hornsey method of total pigment analysis (Hornsey, 1956). Triplicate samples of chopped meat (10 ± 0.1 g) were weighed into 50-mL centrifuge tubes. To this was added about half of an acidified acetone solution made up to contain 40 mL acetone, 9 mL water (taking into account the amount of moisture in the meat), and 1 mL HCl. Each sample was homogenized for 15 s with a Kinematica polytron (Luzern, Switzerland) and the remaining acidified acetone solution was added. The samples were mixed thoroughly, and the tubes were capped tightly and allowed to stand in the dark for at least 1 h before being centrifuged at 2200 g for 10 min. The supernatant was then filtered (GF/A filter paper, Whatman, Maidstone, England) and the absorbance was measured at 640 nm against a reagent blank. The absorbance was multiplied by the factor 6800 and then divided by the sample weight to give the concentration of total pigments in the meat as μg hematin/g meat. The iron content of each sample was calculated with the factor of 0.0882 μg iron/μg hematin (Merck Index, 1989).

**Percent Moisture.** Moisture was determined on each meat purchase by weighing triplicate (~2 g) of ground meat into aluminum weigh dishes and drying in an oven for 18-24 h at 105°C (McNeal, 1990). Moisture lost was divided by initial weight and multiplied by 100.

**Statistical Analysis.** All data were averaged and compared by a balanced analysis of variance program (Statistica-Mac, Stat Soft, Tulsa, OK). When the F values for any mean were significant at p < 0.01, Fisher’s least significant difference (LSD) test was calculated.
RESULTS AND DISCUSSION

Methodology. The Association of Official Analytical Chemists (McNeal, 1990) does not yet have any recommended methods of analysis for the detection of either total, heme, or nonheme iron in meats. The wet digestion method of ashing biological materials using concentrated nitric acid, as used in this study, was the same method that proved to be the most effective by Clegg et al. (1981a, b). In these studies in which both wet- and dry-ashing techniques were used, wet ashing was reported to be superior for the preparation of biological tissues for the analysis of iron. This may be true because iron can be lost during dry ashing by adhesion to vessel walls or by volatilization (Clark, 1976). Clegg et al. (1981a, b) further note that tissues containing high levels of fat would be more efficiently digested with a stronger oxidant, like a nitric-perchloric acid mix. The validity of the total iron procedure employed here was established by analyzing National Institute of Science and Technology reference materials with each total iron analysis. Analyzed values for total iron were statistically equivalent to certified values; that is, 18.4 ± 1.5 μg/g versus 18.3 ± 0.5 μg/g for wheat flour and 192 μg/g ± 12 versus 194 ± 20 μg/g for bovine liver. Recent research (Carpenter and Clark, 1995) indicates that the Hornsey method of heme iron analysis is more accurate, repeatable, and reproducible than nonheme and total iron analyses. Other investigators have also found the Hornsey method (1956) of heme iron analysis to be reliable and repeatable (Buchowski et al., 1988; Krzywicki, 1982; Ladikos and Wedzicha, 1988; Warriss et al., 1988) with minimal sources of contamination, allowing for accurate measurements at low iron concentrations.
### TABLE 6. Heme Iron, Total Iron, Percent Heme Iron, and Moisture in Cooked Light Chicken Meat

<table>
<thead>
<tr>
<th>supplier</th>
<th>heme iron μg/g</th>
<th>total iron μg/g</th>
<th>percent heme %</th>
<th>moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.3</td>
<td>6.5</td>
<td>36</td>
<td>65</td>
</tr>
<tr>
<td>B</td>
<td>1.6</td>
<td>6.1</td>
<td>29</td>
<td>66</td>
</tr>
<tr>
<td>C</td>
<td>1.4</td>
<td>5.8</td>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>D</td>
<td>1.6</td>
<td>6.0</td>
<td>30</td>
<td>63</td>
</tr>
<tr>
<td>E</td>
<td>1.6</td>
<td>8.5</td>
<td>22</td>
<td>69</td>
</tr>
<tr>
<td>F</td>
<td>2.0</td>
<td>6.1</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.7 ± 0.5</td>
<td>6.5 ± 2.0</td>
<td>29 ± 11</td>
<td>65 ± 3</td>
</tr>
</tbody>
</table>

LSD \(^b\) -- -- -- --

\( ^a \) Values are micrograms of iron per gram of meat on a fresh weight basis and are reported as means of four samples, each analyzed in triplicate.

\( ^b \) When the F value was significant at \( p < 0.01 \), Fisher’s LSD was calculated.

**Iron Content of Processed Poultry.** Heme and total iron values for processed light chicken meat (breast) and dark chicken meat (drumstick) are given in Table 6 and Table 7, respectively, and reflect the micrograms of iron per gram of tissue on a fresh weight basis. There were significant differences in the values for heme and total iron between sources of dark chicken meats, but not between sources of light chicken meat. There are probably greater variations in heme iron in red meats from working muscles that are well supplied with blood vessels than in light meats from sedentary muscles, which contain less myoglobin and hemoglobin.
### TABLE 7. Heme Iron, Total Iron, Percent Heme Iron, and Moisture in Cooked Dark Chicken Meat

<table>
<thead>
<tr>
<th>supplier</th>
<th>heme iron (µg/g)</th>
<th>total iron (µg/g)</th>
<th>percent heme (%)</th>
<th>moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.6</td>
<td>13.2</td>
<td>43</td>
<td>66</td>
</tr>
<tr>
<td>B</td>
<td>5.5</td>
<td>11.3</td>
<td>48</td>
<td>66</td>
</tr>
<tr>
<td>C</td>
<td>3.4</td>
<td>11.5</td>
<td>32</td>
<td>63</td>
</tr>
<tr>
<td>D</td>
<td>4.6</td>
<td>13.7</td>
<td>34</td>
<td>64</td>
</tr>
<tr>
<td>E</td>
<td>5.1</td>
<td>11.3</td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>F</td>
<td>5.6</td>
<td>13.8</td>
<td>41</td>
<td>64</td>
</tr>
</tbody>
</table>

**Mean ± SD**

<table>
<thead>
<tr>
<th></th>
<th>heme iron (µg/g)</th>
<th>total iron (µg/g)</th>
<th>percent heme (%)</th>
<th>moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>4.9 ± 1.0</td>
<td>12.5 ± 1.3</td>
<td>40 ± 8</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>LSD b</td>
<td>1.5</td>
<td>1.6</td>
<td>10.7</td>
<td>--</td>
</tr>
</tbody>
</table>

a Values are µg of iron per gram of meat on a fresh weight basis and are reported as means of four samples, each analyzed in triplicate.
b When the F value was significant at p < 0.01, Fisher’s LSD was calculated.

The means for total iron reported in this study for both light and dark chicken meat, 6.5 and 12.5 µg/g, respectively, are similar to the 5 and 10 µg/g reported in food composition tables used in England (McCance and Holland, 1991, p. 5). No heme iron values were given, though this reference acknowledges that dietary iron comes “in two well recognised forms, haem and non-haem,” and that “heme iron is less readily solubilised and absorbed than non-haem iron.” Total iron values for light and dark chicken meat (6 and 12 µg/g, respectively) reported for Australian chicken (Hutchinson et al., 1987) are also closely correlated with those reported here, but again no heme iron values are reported. The food composition tables for the United States, the Agriculture Handbook 8
series (Dickey and Weihrauch, 1988), give a total iron value of 9.1 ± 1.29 μg/g for breaded and fried light chicken meat. This value is higher than that reported here, perhaps because it includes the breading, which probably consisted of iron-enriched flour. The handbook value for dark chicken meat (10.8 ± .3 μg/g) is comparable to that obtained in this study. The USDA values are based on only six samples, and those reported here are based on 24 samples. As with the other references, the U.S. food composition tables also do not give heme iron values for meats. Thus, the heme iron data reported here will be useful in determining values for processed light and dark chicken meat.

**Significance.** Heme iron is much more available than nonheme iron (Björn-Rasmussen et al., 1974). Thus, the percentage of iron that is heme is important in estimating the total bioavailable iron in foods. The 29% heme iron reported here for light chicken meat is significantly less than the 40% used by the Monsen model (Monsen et al., 1978) in calculating the bioavailable iron from a typical American meal, and much lower than the 50 to 60% heme iron calculated for light chicken meat by Cook and Monsen (1976) by the nonheme iron method of Torrence and Bothwell (1968). It is perhaps more reliable to analyze for heme iron directly than to analyze for nonheme iron and subtract its value from total iron.

U.S. consumers are aware of the link between diet and health, as is evident in changing dietary patterns. Fish and chicken, for example, are perceived as leaner and healthier than red meats. Consequently, the consumption of red meat has declined 14% since peaking in 1975, the consumption of poultry has tripled since 1960 (Duewer et al., 1993), and the consumption of fish has increased ~20%. Further, the American public is
becoming more dependent upon processed foods. It is estimated that some 70% of all foods are now processed before consumption (American Medical Association, 1974). Processing degrades the highly bioavailable heme iron into the less available nonheme iron (Schricker and Miller, 1983).

These changing patterns of meat consumption necessitate changes in the Monsen model (Monsen et al., 1978), which assumes that 40% of the iron in MFP is heme iron and that 23% of it is absorbed. Carpenter and Mahoney (1992) estimated that the average percent iron found as heme in MFP is 45%, but also noted that this percentage depends on the proportion of fish and poultry, which have considerably lower heme and total iron concentrations. As the trend toward processed foods continues, it is important to know the content of heme and nonheme iron in all ready-to-eat meat products.

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ABSTRACT

It is important for people to establish and maintain iron homeostasis such that both iron deficiency and iron overload are avoided. Because meats provide a large percentage of the iron in typical western diets, and because meat contains both heme and nonheme iron that is absorbed differently, it is important that dietitians have data regarding the heme and nonheme iron content of meats. Since heat processing alters the ratio of heme and nonheme iron in meats, it is also important that heme and nonheme iron values be obtained from meats as they are prepared for consumption.

INTRODUCTION

Iron is the most widely studied of the trace elements and yet is probably also the most misunderstood (Arthur and Isbister, 1987). Its dietary essentiality is unquestioned, but the optimum quantity needed to replace obligatory losses is still being debated (Conrad et al., 1994). The replacement of daily iron losses is easily measured (Green et al., 1968), but the amount of iron allowed to accumulate in storage without increasing the risk of disease has become a major point of interest (Gordeuk et al., 1994; Weinberg and Weinberg, 1986). Meat diets, iron-fortified diets (whether intentionally fortified or as an adulterant), and those high in iron absorption enhancers give a positive iron balance with a subsequent increase in iron stores (Leggett et al., 1990). In contrast, low meat diets, nonmonotonous vegetarian diets, and diets high in iron absorption inhibitors, seldom if
ever give a negative iron balance (Arthur and Isbister, 1987). They are still capable of maintain iron homeostasis (Craig, 1994).

**Iron Homeostasis.** There are two possible consequences from an iron imbalance, iron deficiency and iron overload, both of which can impair health. Various laboratory tests are used to indicate each of these conditions, but the range of iron sufficiency without increased risk from either deficiency or excess has not been clearly defined; hence, correct interpretation of these laboratory iron status markers is difficult. It has become quite apparent, however, that the range of sufficiency is really much narrower than previously assumed, and that diet contributes to iron overload much more frequently than previously believed (Lauffer, 1991). In evaluating the importance of avoiding either iron deficiency or iron excess, a comparison of the associated consequences is warranted.

**Iron Deficiency.** According to Arthur and Isbister (1987), blood loss is the main cause of iron deficiency and diet is rarely, if ever, the cause. The consequences of iron deficiency apparently are restricted to those exhibiting anemia, since low iron stores without anemia, as seen in vegetarians and athletes, are not detrimental but rather protective against several degenerative diseases (Chang-Claude and Frentzel-Beyme, 1993; Connor et al., 1978; Craig, 1994; Sullivan, 1983). Anemia resulting from insufficient body iron (low hemoglobin count in the erythrocytes) is characterized by reduced work capacity (Viteri and Torun, 1974), impaired neurological functioning (Pollitt, 1993), compromised immunity (Dallman, 1987), impeded gastric activity (Baynes and Bothwell, 1990), and complications of pregnancy (Carriaga et al., 1991). Aside from insufficient iron during gestational development, which generally results in spontaneous
abortion, iron deficiency rarely kills (Emery, 1991). This is not the case with iron overload.

**Iron Overload.** Iron overload is the result of two basic types of iron accumulation, inherited and dietary. Both types were once believed to be rare but are now known to be fairly common (Edwards et al., 1989; Lauffer, 1991). The consequences of either type are the same: an increased risk of cardiomyopathy (Salonen et al., 1992; Sullivan, 1981), arthropathy (Blake et al., 1981; Gordon et al., 1974), neoplasia (Knekt et al., 1994; Stevens et al., 1994), neurological dysfunction (Farrar et al., 1990; Youdim et al., 1993), diabetes mellitus (Phatak and Cappuccio, 1994), accelerated aging (Florence, 1991; Harman, 1982), and microbial infection (Oppenheimer, 1989; Weinberg, 1974). These diseases are chronic, often fatal, and commonly found in developed countries. The percentage of each of these illnesses with an etiology related to iron-mediated free radical production is now being investigated (Lauffer, 1992).

The point at which increasing iron stores become hazardous to health is a prime subject of interest among nutritionists, but regardless of the level of iron stores that are ultimately determined to increase one’s risk of disease, this debate emphasizes the importance of obtaining and maintaining body iron homeostasis. Since the body has a limited capacity to excrete excess iron, iron balance is maintained by absorption rather than excretion (McCance and Widdowson, 1937).

**Iron Absorption.** The quantity of iron in most foods has been determined and reported in numerous publications (Anderson and Hoke, 1990; McCance and Holland, 1991), but has not been reported in its heme and nonheme iron fractions. This is
important because of the differential absorption of each iron type. Nonheme iron is absorbed as an inorganic ion closely controlled by physiological need, whereas meat heme iron is absorbed as an intact molecule with little physiological control (Hallberg et al., 1979). Absorption of both heme and nonheme iron is enhanced by a state of low body iron stores or anemia (Magnusson et al., 1981), but high iron stores inhibit only nonheme iron absorption with little effect upon heme iron absorption (Bezwoda et al., 1983). Before entering the mucosal cells, nonheme iron is also influenced by numerous dietary constituents (Hallberg, 1981), which either enhance or inhibit its uptake. Heme iron, on the other hand, is unaffected by dietary constituents (Callender et al., 1957). Martinez-Torres et al. (1986, p. 1720) stated that “absorption of iron from the heme contained in hemoglobin and myoglobin is little affected by the nature of the meal and even less affected by the iron status of the individual.” Consequently, the absorption of nonheme iron is as low as 2% for individuals with high iron stores and only as high as 20% for individuals with low iron stores who are consuming meals with dietary iron enhancers (Monsen and Balintfy, 1982). Heme iron absorption, on the other hand, is as much as 35% (Monsen, 1988), and is absorbed in direct proportion to quantity in the diet (Bezwoda et al., 1983). Because of this, diets containing heme iron are subject to iron loading (Cook, 1990; Lynch et al., 1989).

**DIETARY MEAT IRON VALUES**

**Heme Iron Levels in Meats.** Since heme is found in the diet as a component of meats, the amount and type of meat consumed is critical in determining the total amount of absorbable iron. Meats from different species have different levels of heme iron
of absorbable iron. Meats from different species have different levels of heme iron (Carpenter and Clark, 1995). Fish and poultry meats have relatively small quantities of heme iron, while red meats have high quantities. There is also variation within species, depending upon the particular muscle selected for consumption (Schricker et al., 1982). White muscle, such as that found in poultry breast meat, is much lower in heme than the dark muscle found in the leg (Clark et al., 1997). Each meat will, therefore, have a different effect upon the amount of iron absorbed and upon the risk of iron accumulation.

The red meats (beef, lamb, and pork) have greater total iron and a greater percent of that total as heme and hence have more bioavailable iron. In contrast, poultry meats have lower heme and total iron with much less actually absorbed. In formulating diets for people with high iron stores, such as mature men and postmenopausal women, red meats would give a positive iron balance and thereby increase the risk of disease. Poultry meat, on the other hand, would probably not increase the body iron burden.

It should also be noted that heat processing breaks the heme ring and releases the heme iron into the nonheme iron pool (Han et al., 1993). This alters the heme-to-nonheme iron ratio and significantly reduces the amount of iron available from that meat (Buchowski et al., 1988).

In addition to the high absorption of heme iron from dietary meat, there is something in meats, commonly called the “meat factor,” that enhances the absorption of nonheme iron (Zhang et al., 1990). This also contributes to the concern about increasing body iron stores. Because of the increase in body iron stores resulting from high meat diets, Conrad et al. (1994) recommended that supplemental iron should not be added to
foods and that meat consumption in western societies should be curtailed. Until the United States follows the lead of Denmark in abolishing their mandatory fortification of flour (Milman et al., 1995), it becomes more important for dietitians to formulate diets designed to maintain iron homeostasis. Nutrient composition tables with heme and nonheme iron data for the various meats normally consumed will greatly help dietitians in achieving this goal.

By using heme and nonheme iron data previously reported (Carpenter and Clark, 1995; Clark et al., 1997), the quantity of each type of iron in a typical serving of various meats was calculated and listed in Table 8. This table also includes total bioavailable iron for each of the meats. These values were calculated by multiplying each iron fraction, heme and nonheme, times its estimated bioavailability as given by Carpenter and Mahoney (1992). Four values are given for each meat, each representing different levels of absorption for persons with iron stores of 0, 250, 500, and 1000 mg.

**Significance.** The main purpose of formulating diets is to maintain nutritional homeostasis. Considering the relatively narrow margin of safety for iron, controlling dietary intake of this essential micronutrient is particularly important. This can be done by adding heme and nonheme iron values, determined on heat-processed meats, to nutrient composition tables currently containing only total iron values. This will give dietitians and other nutritionists the ability to calculate the bioavailability of both heme and nonheme iron with the intent to maintain iron homeostasis, thereby avoiding the detrimental consequences of iron deficiency and especially iron overload.
### TABLE 8. Quantity of Heme, Nonheme, and Bioavailable Iron per 4-Ounce Serving

<table>
<thead>
<tr>
<th>meat</th>
<th>heme iron mg/serving</th>
<th>nonheme iron mg/serving</th>
<th>total bioavailable iron&lt;sup&gt;a&lt;/sup&gt; mg/serving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Beef</td>
<td>2.260</td>
<td>0.960</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.748</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.597</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.377</td>
</tr>
<tr>
<td>Lamb Chop</td>
<td>1.582</td>
<td>0.768</td>
<td>0.707</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.535</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.268</td>
</tr>
<tr>
<td>Pork Round</td>
<td>1.017</td>
<td>0.655</td>
<td>0.487</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.383</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>0.286</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.179</td>
</tr>
<tr>
<td>Cured Pork</td>
<td>0.746</td>
<td>0.441</td>
<td>0.349</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.262</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.207</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.130</td>
</tr>
<tr>
<td>Ground Turkey</td>
<td>0.735</td>
<td>1.119</td>
<td>0.481</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.340</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>0.259</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.155</td>
</tr>
<tr>
<td>Chicken Thigh</td>
<td>0.554</td>
<td>0.859</td>
<td>0.366</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.258</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.117</td>
</tr>
<tr>
<td>Chicken Breast</td>
<td>0.192</td>
<td>0.542</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.050</td>
</tr>
</tbody>
</table>

<sup>a</sup> The four values listed for each meat represent different levels of absorption for persons with iron stores of 0, 250, 500, and 1000 mg (Carpenter and Mahoney, 1992).
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CHAPTER VI
SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

SUMMARY

The first objective of this research, to establish accurate, precise, and specific methods of analysis for quantifying heme, nonheme, and total iron in both fresh and heat-processed meats, was accomplished and reported in Chapter III. This was done by comparing various methods for the recovery of heme, nonheme, and total iron. For heme iron analysis, only the acidified acetone extraction method developed by Hornsey (1956) was tested. This method has already been sufficiently tested by numerous laboratories for many years and found to be accurate, specific, and precise. Two nonheme iron methods were objectively measured, both employing Ferrozine color reagent following extraction by either HCl-trichloroacetic acid or sodium pyrophosphate. Total iron recovery was measured by Ferrozine and atomic absorption spectrometry (AAS) following a wet-ashing procedure using nitric acid and Caro’s reagent. The Caro’s reagent was made fresh before use by adding 4 parts 30% hydrogen peroxide to 1 part sulfuric acid. Methods were tested for the recovery of iron from NIST liver and wheat standards and from lean, fat, and cooked ground beef samples spiked with hemoglobin and ferric chloride.

Total iron methods recovered all the nonheme iron from all samples tested (99±4%; 95% CI) but less than all of the heme iron that had been spiked into fat and cooked ground beef (94±3%; 95% CI). This indicated possible interference from fats and cooking. Undigested fats may have bound the iron and prevented its detection in total
iron analyses, and cooking may have formed conglomerates resistive to acid digestion. Nonheme iron methods recovered all of the nonheme iron, plus significant quantities of heme iron (11±4%; 95% CI) that had been spiked into the meat samples. This indicates that there was a breakage in the porphyrin ring structure and a subsequent release of the heme-bound iron. The heme iron method recovered all of the heme iron spikes (100±4%; 95% CI) but none of the ferric chloride spikes. The coefficient of variation for repeatability was 2% for the heme method, 3.5% for total iron, and 6-8% for nonheme iron. The coefficient of variation for reproducibility ranged from 3% for heme iron, to 3.5% for total iron, to 14-22% for nonheme iron. The heme iron method was the most accurate, specific, and precise of all the methods investigated, while the nonheme iron methods were the least accurate, specific, and precise.

Results of this research indicate that greater accuracy, specificity, and precision could be obtained by analyzing meats for heme and total iron. Nonheme iron values could then be determined by subtracting heme iron from total iron. Either Ferrozine or AAS would give equally reliable results. Faster analyses could, however, be obtained by AAS.

After measuring the accuracy, specificity, and precision of the various methods tested, several fresh and heat-processed meats were analyzed for heme, nonheme, and total iron. Total iron was determined by using Ferrozine to detect the iron in wet-ash digests. The wet-ashing technique was a unique method developed in our laboratory to minimize the interference from fats. Fats are a problem in wet-ash digestions because of the difficulty in breaking down the long-chained fatty acids. Undigested fats form complexes with mineral constituents, thereby giving lower mineral values when analyzed.
Both nonheme iron methods lacked specificity. The method developed by Torrence and Bothwell (1968) was modified by Schricker et al. (1982) to improve detection of nonheme iron, and modified again by Rhee and Ziprin (1987) to minimize the breakage of heme molecules during the heated incubation period. Neither modification, however, eliminated all the problems, as was evident in this research.

Both fresh and heat-processed samples of beef, pork, lamb, chicken, turkey, and cured pork were analyzed. The heat-processing method used for each meat was the one most commonly used by consumers for that particular meat cut. Heating was applied until the internal temperature reached 71°C, the temperature assigned to medium-done meats. Cooking reduces the moisture content of the meats, so iron values were generally higher in heat-processed meats. Total iron content among the different meats varied from a low of 4.3 μg/g with 25% heme for heat-processed chicken breast to a high of over 30 μg/g with about 90% heme in raw beef. This indicates that the Monsen model for determining iron availability (Monsen et al., 1978), which assumes all meats have 40% heme, should be modified to more accurately estimate iron bioavailability. The inaccuracy of the model would be even greater for individuals who consistently prefer red meats over poultry in their diet.

The second objective, to determine the relative amounts of heme and total iron in various beef products in a ready-to-eat condition, was accomplished and reported in Appendix F. The percent of total iron that was heme varied from 30% for beef franks to over 60% for beef burgers and steaks. This indicates that there is great variability in the amount of heme and nonheme iron in the different beef products and illustrates the need
for generating a reliable database of heme and nonheme iron values for the various cuts of meat as prepared for consumption.

The third objective, to determine the relative amounts of heme and total iron in various chicken products in a ready-to-eat condition, was accomplished and reported in Chapter IV. In this study, dark chicken meat taken from the leg was found to have three times as much total iron than that found in light chicken meat taken from the breast. Heme iron percentages were also higher in chicken leg meat. This wide variation in the heme iron content of chicken meat, depending upon where the meat was taken from, would enable poultry meats to be included in diets requiring either high or low iron intake. Such variations in iron values from the same meat animal reaffirms the potential error in trying to oversimplify a formula designed to estimate the availability of a complex mineral like iron (Monsen et al., 1978).

Chapter V accomplished the fourth objective, to provide dietary data of potential iron availability from a variety of meats. Such data can be used in preparing diets designed to establish and maintain iron homeostasis. The ranking of meats from the highest to the lowest bioavailable iron are beef > lamb > pork > turkey > cured pork > chicken thigh > chicken breast. Dietary beef would give a positive iron balance and would possess the greatest risk of iron accumulation. The risk of iron accumulation from most light poultry meats, on the other hand, would be minimal. An awareness of the bioavailable iron in the different meats will enable dietitians to formulate diets designed to meet individual needs while maintaining iron homeostasis.
CONCLUSIONS

Overall results of this research indicate significant variations in total, heme, and nonheme iron contents of the various meats analyzed. There was also significant variation within animal species depending upon the type of muscle. Meat derived from highly active muscles, such as the gluteus medius or biceps femoris, was higher in both total and heme iron. Meat derived from less active muscles, such as the pectoralis major in chicken or the longissimus dorsi in beef, was lower in heme and total iron. Aside from sampling errors, there was also considerable variation within each muscle meat analyzed. This may have been partially the result of the differing effects of heat processing. Samples were obtained on different days and heat processed to a specified internal temperature as measured by an internal probe. Inside and outside portions of the same sample would vary in their degree of doneness, thereby giving different values for heme and nonheme iron. A lack of thorough mixing of the sample before weighing out the correct amount for analysis could account for some of the variation.

A major source of error in the nonheme iron analyses, which probably accounts for much of the variability in this method, was the prolonged exposure to heat. Both nonheme iron methods employed an incubation period of 20 h at 65°C. Though the modification of Rhee and Ziprin (1987) included the addition of sodium nitrite to impede the breakage of the porphyrin ring, the prolonged heat processing was still able to do considerable damage to the ring structure, thereby releasing the heme iron into the nonheme iron pool. Indication for this was obtained by recoveries of greater than 100% for nonheme iron when spiked with hemoglobin.
Considering the possible overestimation of nonheme iron from meats by prolonged incubation in a hot water bath, the water extraction method (Ahn et al., 1993) should be more thoroughly investigated. This method has been criticized to underestimate the true nonheme iron value (Rhee and Ziprin, 1987), but considering the overestimation of the other nonheme iron methods, water extraction could probably be perfected to give more specific results than that evident in extraction methods employing heat.

The total iron values obtained in this research for beef, pork, lamb, chicken, and turkey were similar to values recorded in the USDA Agriculture Handbooks: 8-13 for beef (Anderson and Hoke, 1990); 8-10 for pork (Anderson et al., 1992); 8-17 for lamb (Anderson et al., 1989); and 8-5 for poultry (Posati, 1979). Schricker et al. (1982) reported values similar to those reported here. They noted at the time of their publication that the pork values recorded in the USDA Handbook 8-10 (Watt and Merrill, 1963) were three times higher than the ones they had obtained. The Handbook was updated in 1992 (Anderson et al., 1992) and pork values are now similar to Schricker’s and those we report here (Carpenter and Clark, 1995). These discrepancies, as corrected in Handbook 8-10, suggest the need for accumulating a database of iron values for each species of animal, using standardized methods of analysis. After a range of data is established for each species, any laboratory reporting values outside this range would need to look for an explanation. If the methodology for each iron test is standardized, an explanation for values outside the determined range would be easier to find.
RECOMMENDATIONS

Results obtained in this research on methodologies for heme, nonheme, and total iron analyses are merely the beginning of what must expand into a collaborative study involving many laboratories. We have collaborated with the Meat and Livestock Board and found good agreement with them on total iron analysis in fatty meat tissues (unpublished data), but collaborative research must include common analyses for each of the iron fractions: heme, nonheme, and total. We have established accurate, specific, and reliable heme and total iron analyses for our laboratory, but this must be extended beyond the confines of the Utah State University Department of Nutrition and Food Sciences.

Similarly, the database begun on various beef and chicken preparations must be expanded to include all meats normally consumed. All the different pork and lamb preparations, for example, should be analyzed for heme and total iron in a ready-to-eat condition. All the different seafood preparations also need to be analyzed for heme and total iron and included in the database on iron composition of meat products. Since the methods of heat processing (length of time and temperature) have such a profound effect upon the different proportions of heme and nonheme iron in meats (Han et al., 1993), more research is needed to elucidate these effects.

One practical application of the data accumulated in this research would be in revising the USDA Agriculture Handbook 8 series of booklets containing food composition tables. The food composition tables for meats in these booklets give only total iron values. Because of the differential absorption of heme and nonheme iron from the diet and the importance of maintaining iron homeostasis, these total iron values need
to be partitioned into heme and nonheme iron values. If absorption of heme and nonheme iron were the same, total iron values would be adequate in food composition tables and reporting heme and nonheme iron values would merely be academic.

Heme and nonheme iron data are useful then in formulating rational diets to assure iron homeostasis. Since nonheme iron absorption is closely controlled by body iron status but heme iron absorption is not, the greatest concern in regards to iron intake is the amount of heme iron in the diet. With minimal body control of heme absorption, diets rich in heme would contribute to a positive iron balance. This positive iron balance would soon lead to excess iron stores and a subsequent increase in the risk of many degenerative diseases. This emphasizes the need to control heme intake, which, aside from heme fortification of grain products (Walter et al., 1993), is solely contained in animal flesh, MFP. There is a need, therefore, to be able to evaluate MFP as to the degree of risk each meat cut poses for iron accumulation. Chapter V is an attempt to satisfy the need of evaluating meats as to the degree of risk of body iron buildup.

In a state of iron demand, such as pregnancy or heavy athletic activity, consumption of red meats, with high bioavailable iron, can be increased. On the other hand, in a relatively inactive state, red meat consumption should be done sparingly, and then accompanied by inhibitory substances, such as phytates and tannins found in whole grains and seeds. Fish and poultry can also be consumed in higher quantities while reducing the consumption of red meats, since they have lower bioavailable iron and pose less or no risk of iron accumulation.

The prevention of iron accumulation and the avoidance of the tragic consequences
are centered in a knowledge of the dark side of iron. Researchers may continue to question the source of high body iron or the level of stored iron that is safe, but they can no longer question the results of iron-mediated free radical production resulting in cellular and organ damage (Florence, 1991; Halliwell and Gutteridge, 1984; Lauffer, 1992).

Prevention of these consequences is as simple as minimizing the consumption of iron-dense diets (Conrad et al., 1994), avoiding iron-fortified foods (Crosby, 1986), reducing or eliminating the consumption of alcoholic beverages (Duane et al., 1992), reducing the use of iron enhancers with meals, such as fats (Simpson et al., 1988), sugars (Charley et al., 1963), and ascorbate (Siegenberg et al., 1991), avoiding the use of iron cookware, especially Dutch ovens (Cheng and Brittin, 1991), and incorporating iron inhibitors in meals with meats (Empson et al., 1991; Tuntawiroon et al., 1991).

One of the primary inhibitors of iron absorption, which has for years been grossly maligned in this regard, is phytate. Phytate is an important constituent of fiber, found in whole grains and seeds. It has long been recognized for some of its beneficial functions in bowel activity (Kelsay, 1981). It decreases the transit time of the fecal mass through the intestines and helps prevent impaction in the elderly. Phytate has also been shown to suppress colonic cancer (Graf and Eaton, 1985; Shamsuddin et al., 1988), to reduce the risk of breast cancer (Lee et al., 1991), to decrease the formation of kidney stones, to lower blood cholesterol (Empson et al., 1991), to lower serum lipid concentrations (Jariwala, 1992), and to reduce the rate of dissolution of tooth enamel (Magrill, 1972). Furthermore, it is being recognized as a natural antioxidant. It suppresses lipid peroxidation by blocking iron-driven hydroxyl radical production (Graf et al., 1987). The
antioxidant features of phytic acid have also been tested in food preservation. For
example, 1 mM phytic acid protected against oxidative damage and extended shelf life
four-fold in an oil-in-water emulsion. It is, therefore, being recommended as a natural
preservative for meats and other foods. In poultry, phytic acid substantially inhibited the
uptake of oxygen, malondialdehyde formation, and the development of warmed-over
flavor (Empson et al., 1991). Phytate, like selenium, will likely undergo a transition from
detrimental dietary constituent to beneficial nutrient, and attempts to include it in the diet
will be encouraged to avoid iron excess instead of discouraged because of its mineral­
binding capabilities and fear of deficiency.

In comparing iron excess with iron deficiency, Sullivan (1992) reported that stored
iron is not essential for life or preventing anemia. Emery (1991) stated that fatigue, one of
the primary clinical symptoms of anemia, rarely kills, but that infection, stimulated by iron
availability, often does. He further stated that mortality from iron deficiency during the
first few months of infant life is virtually unknown but the primary cause of infant
mortality is gastrointestinal infections (Emery, 1991). Gastrointestinal infections are
facilitated by intestinal iron derived from iron-fortified diets. The supplemental iron
supplied to infants is added to that supplied by the rapid breakdown of fetal hemoglobin.
Supplemental iron early in life saturates the iron-sequestering milk protein, lactoferrin, and
overcomes the naturally iron-poor environment of the infant's intestines. This will then
allow the establishment and proliferation of putrefactive and pathogenic microorganisms,
which leads to the gastrointestinal infections described by Emery (1991) as the primary
cause of infant mortality.
In recognition of the hazards of excess iron, some physicians are now recommending widespread screening programs to identify people with high iron stores (Herbert, 1992). Phlebotomy is used extensively for patients with hemochromatosis, and a regular blood donation program would also be advised for all others exhibiting high iron stores, such as men and postmenopausal women. Casale et al. (1983, p. 398) reported a higher “survival rate...for blood donors than for non-blood donors, especially between 50 and 70 years of age.”

If, indeed, iron deficiency has been “misunderstood, misdiagnosed, and mistreated,” and is rarely the result of inadequate dietary iron absorption, but rather the result of blood loss (Arthur and Isbister, 1987), then the focus of public attention and university research should be on ways to minimize the hazards of iron excess, to strengthen the iron-withholding defense during episodes of microbial infection, and to control the quantities of iron to which we are all exposed, and not on iron deficiency. The consequences of a positive iron balance leading to iron excess, in contrast to the consequences of a negative iron balance leading to iron deficiency, are so much more devastating and damaging that it is paramount for a paradigm shift to take place in our concept of iron in human nutrition. The apparent narrow margin of safety for iron that is evident from recent research reports revealing iron-mediated Fenton chemistry, brought about by excess body iron, can no longer be ignored.

LITERATURE CITED


Appendix A

Heme Iron Method of Analysis

- Do all procedures in subdued light.
- Trim meat of all visible fat and chop finely.
- Weight 2 g aliquots into aluminum weigh dishes for moisture determinations.
- Dry samples to constant weight, reweigh, and calculate % moisture.
- Weight 10 g aliquots into 50 ml centrifuge tubes with screw caps.
- Prepare just enough extraction mixture for each sample: 40 parts acetone, 9 pts water (includes meat water), 1 pt conc HCl.
- Add part of 50 ml extraction mixture to one tube and polytron for 30 sec.
- Add remainder of 50 ml extraction mixture, cap, mix, and put in the dark.
- After one hour centrifuge for 10 min at 2000 x g.
- Filter slurry through GF/A glassfiber filter paper into 13 x 100 mm test tubes.
- Measure absorbance in 1 cm cells at 640 nm against a reagent blank.
- Calculate hematin by (absorbance x 680) and standardize weight (10/weight).
- Calculate % heme iron (adjusted hematin x 8.85%)

Described from Hornsey (1956).
Nonheme Iron Method of Analysis (Pyrophosphate)

- Trim meat of all visible fat and chop finely.
- Weight 2 g aliquots into aluminum weigh dishes for moisture determinations.
- Dry samples to constant weight, reweigh, and calculate % moisture.
- Weigh 2 g aliquots into 50 ml polyethylene centrifuge tubes with screw caps.
- Prepare reagent blanks by adding 3 ml DD water to 3 centrifuge tubes.
- Prepare just enough extraction mixture for each sample: 5 ml pyrophosphate and 10 ml 10% TCA per sample.
- Add 15 ml extraction mixture and homogenize for 15 sec with a polytron.
- Stopper tightly and incubate in shaker water bath at 55 °C for 18 hours.
- Centrifuge at 2000 x g for 20 min, put 1 ml clear supernatant into 13 x 100 tubes.
- Prepare Fe standards by serial dilution (8 µg Fe/ml to 0.5 µg/ml), treat as sample.
- Add 1 ml of 1% ascorbic acid, shake vigorously and let stand 15 min.
- Add 2 ml 10% ammonium acetate, (bring pH to 5 with NH₄OH if needed).
- Add 1 ml 1.0 mM ferrozine and let stand 30 min.
- Measure absorbance in 1 ml cell at 562 nm against a reagent blank.
- Prepare standard curve of concentrations versus absorbance.
- Calculate nonheme iron in sample: (concentration x moisture / weight).

Appendix C

Nonheme Iron Method of Analysis (HCl-TCA)

- Trim meat of all visible fat and chop finely.
- Weight 2 g aliquots into aluminum weigh dishes for moisture determinations.
- Dry samples to constant weight, reweigh, and calculate % moisture.
- Weigh 5 g aliquots into 50 ml polyethylene centrifuge tubes with screw caps.
- Prepare reagent blanks by adding 5 ml DD water to 3 centrifuge tubes.
- Add 0.2 ml of 0.4% sodium nitrite to each tube and let stand for several hours.
- Prepare just enough extraction mixture for each sample: Equal parts of 6N HCl and 40% trichloroacetic acid (TCA).
- Add 15 ml extraction mixture and homogenize for 15 sec with a polytron.
- Stopper tightly and incubate in shaker water bath at 55 °C for 18 hours.
- Centrifuge at 2000 x g for 20 min, put 1 ml clear supernatant into 13 x 100 tubes
- Prepare Fe standards by serial dilution (8 µg Fe/ml to 0.5 µg/ml), treat as sample
- Add 1 ml of 1% ascorbic acid, shake vigorously and let stand 15 min.
- Add 2 ml 10% ammonium acetate, (bring pH to 5 with NH₄OH if needed).
- Add 1 ml 1.0 mM ferrozine and let stand 30 min.
- Measure absorbance in 1 ml cell at 562 nm against a reagent blank.
- Prepare standard curve of concentrations versus absorbance.
- Calculate nonheme iron in sample: (concentration x moisture / weight).

Described by Rhee and Ziprin, 1987, and modified for use with ferrozine.
Appendix D

Total Iron Method of Analysis (Ferrozine)

- Trim meat of all visible fat and chop finely.
- Weigh 2 g aliquots of meat into 250 ml Erlenmeyer flasks.
- Set aside 3 flasks for reagent blanks.
- Add 15 ml conc nitric acid and let predigest for several hours or overnight.
- Put on hot plate at 100°C until dry.
- If ash is black add 5 ml more nitric acid and dry until ash is brown or yellow.
- Prepare Caro’s acid by mixing 4 parts H₂O₂ to 1 part conc H₂SO₄.
- Turn up heat and add Caro’s acid 1 ml at a time until solution remains clear.
- Transfer to volumetric flask and bring to the mark with DD water.
- Add 1 ml of sample to 13 x 100 tubes, (bring pH to 5 with NH₄OH if needed)
- Prepare Fe standards by serial dilution (8 μg Fe/ml to 0.5 μg/ml), treat as sample
- Add 1 ml of 1% ascorbic acid, shake vigorously and let stand 15 min.
- Add 2 ml 20% ammonium acetate.
- Add 1 ml 1.0 mM ferrozine and let stand 30 min.
- Measure absorbance in 1 ml cell at 562 nm against a reagent blank.
- Prepare standard curve of concentrations versus absorbance.
- Calculate total iron in sample: (concentration x dilution factor / weight).

Developed from procedures by Hach et al., 1985; Clegg et al., 1981; Carter, 1971.
Appendix E

Total Iron Method of Analysis (Atomic Absorption)

- Trim meat of all visible fat and chop finely.
- Weigh 2 g aliquots of meat into 250 ml Erlenmeyer flasks.
- Use 3 flasks for reagent blanks and 5 flasks for standards, treat as samples.
- Add 15 ml conc nitric acid and let predigest for several hours or overnight.
- Put on hot plate at 100°C until dry.
- If ash is black add 5 ml more nitric acid and dry until ash is brown or yellow.
- Prepare Caro’s acid by mixing 4 parts $\text{H}_2\text{O}_2$ to 1 part conc $\text{H}_2\text{SO}_4$.
- Turn up heat and add Caro’s acid 1 ml at a time until solution remains clear.
- Transfer to volumetric flask and bring to the mark with DD water.
- Prepare Fe standards in 5 reagent flasks (8 $\mu$g Fe/ml to 0.5 $\mu$g/ml).
- Set up atomic absorption spectrophotometer.
- Calibrate atomic absorption spectrophotometer.
- Measure concentrations of samples.

Developed from procedures by Hach et al., 1985; Clegg et al., 1981; Carter, 1971.
HEME IRON CONTENT IN SELECTED READY-TO-SERVE BEEF PRODUCTS

P.V. Mrudula Kalpalathika, Eli M. Clark, and Arthur W. Mahoney

Department of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322

ABSTRACT

Selected ready-to-eat meat foods, e.g., beef burger, frankfurter, beef steak, and roast beef, from fastfood restaurants and groceries were analyzed for total iron, heme and nonheme iron contents. The different meat products contained similar amounts of total iron. The heme iron contents (ready-to-serve basis) ranged from 16.0 to 26.9 μg/g for beef burger, from 6.2 to 14.6 μg/g for frankfurter, from 13.4 to 36.3 μg/g for beef steak, and from 11.3 to 23.7 μg/g for roast beef. Percentage heme iron ranged from 50.2 to 63.8% for these meat foods. Total iron, heme iron, percentage heme iron, and nonheme iron varied significantly (P < 0.01) among meat foods, sources, and replications.

INTRODUCTION

Dietary heme iron is important in iron nutrition because factors like phytates do not interfere with its absorption (Bezwoda et al., 1983; Brown et al., 1968; Conrad et al., 1966; Hallberg and Sölvell, 1967) and it is much better absorbed (>15%) than nonheme...

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iron (<5%) (Hussain et al., 1965; Layrisse et al., 1974; Schricker et al., 1982). Monsen et al. (1978) assumed that 40% of the iron in meat, fish, and poultry is heme and that 23% of dietary heme and 2-8% of dietary iron is absorbable. On this basis, they calculated absorbable dietary iron and noted that predicting the dietary iron availability depends on accurate estimates of heme and nonheme iron. However, there is limited information regarding the heme iron content of meat products as consumed (ie., generally cooked). The available data are largely for raw meat, and the determined values vary widely. Since heat destroys heme iron (Igene et al., 1979; Schricker and Miller, 1983; Jansuittivechakul et al., 1985; Buchowski, et al., 1988; King et al., 1990) it is inaccurate to estimate the heme iron content of cooked meat foods on the basis of analysis of raw meat. Cooking time and source of meat affect heme iron content (Hendricks et al., 1987; Buchowski et al., 1988) Thus, it would be more accurate to estimate absorbable dietary iron intakes on the basis of actual heme iron contents of the different meat, fish, and poultry products ready-to-serve.

Not much information is available on the heme iron and nonheme iron content of ready-to-serve meat products such as beef burger, frankfurter, beef steak, and roast beef. The objectives of this study were (1) to quantify the total iron, heme iron, and nonheme iron content in ready-to-serve meat products, (2) to calculate the percentage of meat iron that is heme, and (3) to compare the different iron forms in meat products from different sources and prepared in different ways.
MATERIALS AND METHODS

Sample Preparations. All the meat samples were purchased ready-to-serve from fast-food restaurants or grocery stores and analyzed for moisture and heme iron on the day of purchase. Beef burger was sampled from 6 sources; frankfurter from 3 sources; beef steak from 4 sources; and roast beef from 10 sources (n = 30 for all meat products except for steak, for which n = 32). The meat samples were collected from Nov 23, 1988 through May 2, 1989. The samples were weighed, chopped finely, packed in aluminum pouches, and kept in the dark until analysis.

Chemical Analyses. All the analyses were done in triplicate for each sample.

Total iron (TFe) was determined in wet ashed samples by using ferrozine. Wet ashing involved digesting 1-g aliquots in boiling concentrated nitric acid until the solution was clear and all yellow fumes were dissipated. Then, several drops of 30 % hydrogen peroxide was added to each sample and heating was continued until a white ash remained. The ash was dissolved in 6 N hydrochloric acid and diluted to 5 mL with deionized water. To 1 mL of neutralized ash solution (pH adjusted to 8 with 12 N NH₄OH) was added 1 mL of 10% hydroxylamine hydrochloride and the solution was mixed and allowed to stand for 15 min. To this was added 1 mL of 10% ammonium acetate, the solution was mixed, and 1 mL of 1 μM ferrozine was added. The volume was made to 5 mL, and the solution was mixed and left to stand in the dark for 45 min. The absorbance was determined at 562 nm, and the concentration of total iron in the samples was determined against reference standards. With each batch of meat samples, duplicate samples of the National Institute of Science and Technology (NIST, formerly the National Bureau of Standards)
bovine liver (1577a) and wheat flour (1567) standard reference materials (SRMs) were analyzed for total iron as a control for total iron determination. Across all batches, analyzed values for the bovine liver and wheat flour SRMs averaged (mean ± SD) 202 ± 11.8 and 19 ± 1.7 μg/g, respectively, as compared with certified values of 193 ± 20 and 18.3 ± 1.0 μg/g, respectively.

*Heme iron* (HFe) was determined in acid-acetone extracts of the samples by the Hornsey (1956) method. Approximately 10-g aliquots were made into a smooth paste with 10 mL of acid-acetone mixture (40 mL of acetone, 4 mL of water, and 1 mL of concentrated hydrochloric acid). Then, an additional 35 mL of acid-acetone mixture was added, and the contents were mixed well and kept in the dark for 1 h. The extract was centrifuged at 2200 g at 10°C for 10 min. The supernatant was filtered through glass microfiber filters (Whatman GF/A). This gave a solution of acid hematin in 80% acid-acetone. Total heme pigment was measured at 640 nm. Total heme as hematin in each sample was calculated as follows:

hematin, g = OD at 640nm x 680 (extinction coefficient) x aliquot wt, g / 10

The HFe content in each sample was then computed by assuming that hematin contains 88.2 mg iron / g as follows:

HFe, μg/g = (hematin, g x 88.2 mg Fe/g) ÷ aliquot wt, g

*Nonheme iron* (NHFe) content of each aliquot was computed as the difference between TFe and HFe as follows:

NHFe, μg/g = TFe, μg/g - HFe. μg/g

*Percent heme iron* (HFe%) was calculated as follows:
\[ \text{HFe\%} = \left( \frac{\text{HFe}, \mu g/g}{\text{TFe}, \mu g/g} \right) \times 100 \]

Percent dry matter (DM\%) in the meat samples was determined on 2 g aliquots by lyophilizing to constant weight (24 h) in a freeze dryer with shelf temperature set at 40°C.

Statistical Analysis. The data were analyzed by using an unbalanced nested analysis of variance (Steel and Torrie, 1980) with four meats (30 replicates samples of beef burger, frankfurter, and roast beef and 32 replicates of beef steak) selected from 3 to 10 sources (frankfurter 3, beef steak 4, beef burger 6, and roast beef 10).

RESULTS AND DISCUSSION

The object of this study was to determine the heme iron content of different ready-to-serve meat products. It is important that correct estimates of food heme iron and nonheme iron content be available for accurate prediction of absorbable dietary iron. TFe, HFe, NHFe, and HFe\% contents of beef steak, beef burger, roast beef, and frankfurter are presented in Table 9. TFe contents were similar among the different meat products. A comparison of the mean TFe values for meats in this study with published values (Richardson et al., 1980; Anderson et al., 1986) leads to some interesting similarities and variations. TFe for beef steak in this study (Table 9) is similar to 35.8 \( \mu g \) Fe/g (for beef, short loin, tenderloin, separable lean only, prime cooked, broiled) reported by Anderson et al. (1986). The TFe values for beef burger, roast beef, and frankfurters are higher than the respective reported values of 24.5 \( \mu g \) of Fe/g (for beef, ground, lean, cooked, broiled, well done; Anderson et al., 1986), 29.4 \( \mu g \) Fe/g (for beef, round, separable lean only, all grades, cooked, roasted; Anderson et al., 1986) and 13.2 \( \mu g \pm 0.3 \) \( \mu g \) of Fe/g (for...
frankfurter, beef; Richardson et al., 1980). We have no explanation for the differences in TFe found in this study as compared with reported TFe values, especially for frankfurters. In this study, analyzed TFe values for NIST bovine liver and wheat flour were consistently within the certified values for these SRMs which were analyzed with each batch of the different meat samples. About 6 months after completing the frankfurter TFe analysis, we analyzed another batch of three frankfurter samples, and these TFe values also averaged 33.0 μg of Fe/g. We therefore feel that the meat TFe values in this study are accurate.

The HFe contents of ready-to-serve beef burger, frankfurter, beef steak, and roast beef are presented in Table 9. The frankfurter HFe level is similar to the 9.1 ± 0.8 μg of Fe/g (mean ± SE) reported by Hendricks et al. (1987) on six frankfurter and bologna samples. Jansuittivechakul et al. (1985) reported 58.3 μg/g of heme iron in lyophilized beef round, baked medium (roast beef), which is similar to the 51.4 μg/g dry matter obtained in this study.

HFe% values for ready-to-serve beef burger, frankfurter, beef steak, and roast beef sampled from various sources (Table 9) are similar to published values. Martinez-Torres et al. (1986) reported HFe% values of 55, 53, and 58%, respectively, for burger, steak, and roast beef. Cook and Monsen (1976), Field et al. (1980), Schricker et al. (1982), Jansuittivechakul et al. (1985), and Buchowski et al. (1988) reported that HFe% ranged from 50 to 60% in beef. In all of the studies cited above, small numbers of samples were analyzed and the methods of meat preparation were highly variable.
### TABLE 9. Total Iron (TFe), Heme Iron (HFe), Nonheme Iron (NHFe), Percent Heme Iron (HFe%), and Dry Matter (DM) in Beef Steak, Beef Burger, Roast Beef, and Frankfurters from Different Sources

<table>
<thead>
<tr>
<th>Sources</th>
<th>TFea</th>
<th>HFea</th>
<th>NHFea</th>
<th>HFe%</th>
<th>DM%</th>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>25.0</td>
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<td>20.8</td>
<td>9.9</td>
<td>67.2</td>
<td>41.2</td>
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<td><strong>Beef Burger</strong></td>
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<td></td>
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<td></td>
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<td>1</td>
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<td>12.8</td>
<td>63.3</td>
<td>46.8</td>
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<td>49.8</td>
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<td>overall mean ± SE</td>
<td>31.5 ±0.6</td>
<td>20.0 ±0.4</td>
<td>11.4 ±0.4</td>
<td>63.8 ±1.0</td>
<td>47.7 ±0.6</td>
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<tr>
<td><strong>Roast Beef</strong></td>
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<td>16.3</td>
<td>19.7</td>
<td>45.3</td>
<td>27.7</td>
</tr>
<tr>
<td>overall mean ± SE</td>
<td>33.6 ±1.0</td>
<td>16.8 ±0.5</td>
<td>16.8 ±0.7</td>
<td>50.2 ±1.1</td>
<td>32.6 ±1.2</td>
</tr>
<tr>
<td><strong>Frankfurter</strong></td>
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<td>25.5</td>
<td>8.6</td>
<td>16.7</td>
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</tr>
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<td>21.6</td>
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<td>47.6</td>
</tr>
<tr>
<td>overall mean ± SE</td>
<td>31.4 ±1.1</td>
<td>10.5 ±0.5</td>
<td>21.0 ±0.7</td>
<td>33.0 ±0.8</td>
<td>46.3 ±1.3</td>
</tr>
</tbody>
</table>

* Micrograms per gram on fresh weight basis.  
  " Each value is a mean of 8 samples analyzed in triplicate.  
  Each value is a mean of 5 samples analyzed in triplicate.  
  Each value is a mean of 3 samples analyzed in triplicate.  
  Each value is a mean of 10 samples analyzed in triplicate.
NHFe contents varied among the meats; it is high for frankfurters and roast beef as compared with beef burger and beef steak (Table 9). Igene et al. (1979), Buchowski et al. (1988), and King et al. (1990) demonstrated that nonheme iron is increased and heme iron is decreased due to heating. It is customary to heat-process frankfurter and roast beef for a longer time, which may account for the increase in NHFe and decrease in HFe% of these products as compared with burger and steak.

One objective of this study was to examine popular meat foods for TFe and HFe%. Table 10 provides mean squares and F values from the analysis of variance for estimates of different iron forms. Mean TFe, HFe, NHFe, HFe%, and DM% were significantly different (p < 0.01) among meat foods and their sources. However, replication mean squares were low for all parameters, indicating that sample variation within a source was relatively small.

From this study, it is clear that red meat has a high level of heme iron and therefore can be expected to contribute much absorbable iron to diets. From this study, burger and steak contain at least 60% heme iron. Because the bioavailability of heme iron is high (23%) as compared with nonheme iron (2-8%) (Monsen et al., 1978), more research on heme content and HFe% in a variety of meat, poultry, and fish products is being conducted to accurately estimate the absorbable dietary iron contributions of these foods.
TABLE 10. Mean Square and F (in Parentheses) Values for Effects of Meat, Source of Meat, and Replication of Samples on Iron Contents of Selected Meat Foods

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>TFe</th>
<th>HFe</th>
<th>NHFe</th>
<th>HFe%</th>
<th>DM%</th>
</tr>
</thead>
<tbody>
<tr>
<td>meat</td>
<td>3</td>
<td>75.6</td>
<td>342.7</td>
<td>251.0</td>
<td>3088</td>
<td>1073</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.64)\textsuperscript{NS}</td>
<td>(6.73)\textsuperscript{b}</td>
<td>(8.09)\textsuperscript{b}</td>
<td>(27.7)\textsuperscript{b}</td>
<td>(15.1)\textsuperscript{b}</td>
</tr>
<tr>
<td>source/meat</td>
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<td>117.7</td>
<td>50.9</td>
<td>31.0</td>
<td>111.4</td>
<td>71.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.02)\textsuperscript{b}</td>
<td>(8.49)\textsuperscript{b}</td>
<td>(3.12)\textsuperscript{b}</td>
<td>(4.85)\textsuperscript{b}</td>
<td>(3.07)\textsuperscript{b}</td>
</tr>
<tr>
<td>replicates/source</td>
<td>99</td>
<td>19.6</td>
<td>6.0</td>
<td>9.9</td>
<td>23.0</td>
<td>23.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Meat foods are beef burger, frankfurter, beef steak, and roast beef. NS, not statistically significant.

\textsuperscript{b} Values carrying the same superscript within rows are significant at p < 0.01.
LITERATURE CITED


Buchowski, M.S.; Mahoney, A.W.; Carpenter, C.E.; Cornforth, D.P. Heating and the distribution of total and heme iron between meat and broth. *J. Food Sci.* 1988, 53, 43.


APPENDIX G

Permission to Reprint Copyrighted Materials

Eldred M. Clark
801 Douglas Dr.
Brigham City, UT 84302
(801) 723-6259

American Chemical Society
c/o Arleen Cortney
1155 16th Street
Washington, D.C. 20036

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Eldred Clark
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Brigham City, UT 84302

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Assistant Copyright Administrator

RE: Your letter dated April 30, 1997

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APPENDIX H

Clarification of Authorship

I acknowledge that although I am the first and third author, respectively, on the following articles, Eldred Clark was the major contributor to the research reported and to the writing of the articles. I give my permission for the articles to be included in his PhD dissertation.


Charles E. Carpenter
Name

Signature

Date 5-13-97
CURRICULUM VITAE

Eldred "Eli" M. Clark
(April 1997)

CAREER OBJECTIVE

To represent the nutritional interests of a pharmaceutical company or food
processor, functioning in quality control and nutritional education.

EDUCATION

Ph.D. in Nutrition & Food Sciences, Utah State University, Logan, UT.

M.S. in Agronomy & Horticulture, Brigham Young University, Provo, UT.

B.S. in Animal Science, minor in Chemistry, Brigham Young University, Provo, UT.

EXPERIENCE

Quality Assurance Chemist, Fresenius Medical Care, Ogden, UT.

Presently work in quality control for renal products, operate laboratory instruments,
and validate instruments and procedures.

Consultant for Lab Support, (On Assignment, Inc.), Ogden, UT; Lewisberry, PA.

Developed nutrition labeling, wrote SOPs & GMPs for laboratory work, validated
laboratory instruments, taught lab personnel SOPs for instrument operations.

Teaching/Research Assistant, Utah State University, Logan, UT.

Developed techniques for iron analyses of biological tissues. Developed labs and
curriculum for plant science classes. Taught classes in forages and crop production.

Wrote $100,000 proposal for iron analysis methodologies.

Assistant Professor, Southern Utah University, Cedar City, UT.
Taught courses in soils, plant sciences, animal sciences, horticulture, and economics. Developed laboratory experiments for soil and plant science classes. Received federal funding on proposal written for curriculum reform. Performed independent research on animal waste recycling. Organized & advised the Agronomy Club. Advised and traveled with the Rodeo Team. Chaired Vocational Agriculture Day activities for Utah high school students for 5 years. Established FFA in local H.S. Environmental Technician, Coronado Generating Station, St. Johns, AZ. Developed environmental monitoring systems for air and water monitoring. Set up, programmed, and operated environmental monitoring equipment. Developed computerized reporting methods for laboratory data. Developed QA/QC protocol for laboratory analyses. Attended special training on quality control methodologies. Researched and published on zero discharge of plant waste waters. County Extension Agent, Utah County, Provo, UT. Advised the public on agronomic and horticultural problems, prepared and delivered speeches on home gardening and horticultural problems, prepared reports.

PUBLICATIONS & PRESENTATIONS


