5-1991

Determining the Validity of Methods Used in Meat Iron Analysis

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DETERMINING THE VALIDITY OF METHODS USED IN MEAT IRON ANALYSIS

by

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

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

1991

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to my major professor, Dr. Arthur W. Mahoney, for his guidance and encouragement during my research work and in the preparation of this thesis.

I extend my gratitude to Dr. Deloy G. Hendricks for his invaluable assistance and his good humor, which always cheered me up.

Sincere thanks are extended to Dr. James Gessaman for his helpful suggestions and willingness to serve on my committee.

I thank my colleagues for their technical help in the laboratory and my friends in Logan for making me feel at home. Special thanks to my dear friend, Hrushi, and my cousin, Soni, for their love and support.

Finally, I thank my parents, Shri. U. Pattabhi Ramiah, and Smt. U. Sowbhagyavathy, for inspiring and stimulating my interest in learning and for their love and constant encouragement. I am grateful to my brothers, Vijay and Vinod, and my sister, Usha, who encouraged me in my early learning experiences.

Padmashri Ummadi

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ABSTRACT

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by

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The validity of the Hornsey method for heme iron, modified Schricker and sodium pyrophosphate extraction methods for nonheme iron and atomic absorption spectrophotometry (AAS) and ferrozine methods for total iron were determined using spikes of hemoglobin, ground beef baked to different degrees of doneness, proportional beef liver:catfish mixtures and National Institute of Science and Technology reference materials.

The mean spike recoveries of 0.01g and 0.02g Hb in raw beef and raw chicken samples were 96.7% of the heme iron for the Hornsey method, 97.9% of the total iron for the ferrozine method, and 85.7% of the total iron for the AAS technique.

In ground beef patties baked rare, medium and well-done, the nonheme iron values increased with doneness as determined by both methods and there were no significant differences between the mean values obtained by the two methods. The sum of heme and nonheme iron equalled total iron in beef patties baked rare and medium. The Hornsey

method seemed to overestimate the heme iron content in well-done beef possibly due to browning. The total iron content remained constant. However, total iron determined by the ferrozine method was higher ($p<.05$) than that analyzed by the AAS method.

Heme and nonheme iron values were plotted against beef liver concentrations in the beef liver:catfish mixtures, and the correlation coefficients obtained were 0.994 for the Hornsey method, 0.991 for the modified Schricker method, and 0.995 for the sodium pyrophosphate method. Heme iron plus nonheme iron equalled total iron for all the mixtures. Student's t test revealed no significant difference between ferrozine total iron values and NIST-certified concentrations, but the AAS total iron values were significantly ($p<.05$) lower than the NIST-certified values.

The Hornsey method was validated for all samples except well-done beef. The two nonheme iron methods were reliable and accurate. While the ferrozine technique was consistent, reliable and accurate, the AAS method was able to detect, on an average, only 80-85% of the total iron present. There was no interference of the sample mineral matrix with the detection ability of the AAS method.

(80 pages)

INTRODUCTION

Iron is a trace element that is a continuing public health concern. Iron deficiency is probably the most common organic disorder seen in clinical medicine; its incidence varies widely with age, sex, race and economic status (Fairbanks et al., 1971; DeMaeyer, 1985; Stoskman, 1987; Arthur and Isbister, 1987; Skikne, 1988). Nutritionists have routinely expressed concern that the levels of iron intake are not adequate and marginally deficient intakes may exist (Wolf, 1982; Arthur and Isbister, 1987). The prevalence of anemia is estimated at 30% of the world population (DeMaeyer and Adiels-Tegman, 1985).

Food iron may appear in several different forms. Elemental iron is used as a food additive. Soluble, complexed, and ionic iron, as either ferrous or ferric iron, may occur in varying amounts depending upon the chemical interactions with food anions and food ligands (Lee and Clydesdale, 1979). Dietary iron is divided into two distinct compartments, heme and nonheme iron, because of their separate absorption pathways from the lumen to the mucosal cell (Hallberg et al., 1979; Cook, 1983).

The heme proteins consist of an iron porphyrin ring and a protein unit. Heme iron is absorbed directly as the intact iron porphyrin complex, and its iron is freed in the intestinal mucosal cell (Turnbull et al., 1962; Bothwell et al., 1979; Cook, 1983). Absorption of iron in hemoglobin, in contrast to that of nonheme iron, is not affected by blocking substances in food (Hallberg, 1981; Bezkorovainy, 1989), reducing agents, ascorbic and hydrochloric acids and synthetic chelating agents which bind ionic iron (Turnbull et al., 1962; Cook, 1983). The rate of iron uptake into the intestinal mucosal cell is increased in iron deficiency (Bezkorovainy, 1989); the biological utilization of the absorbed iron is affected by food source and processing (Kalpalathika et al., 1991). An individual having no iron stores absorbs approximately 35% of the heme iron when ingested as meat; an individual having sufficient iron stores absorbs approximately 25% (Monsen et al., 1978). Analysis of a variety of foods has shown that approximately 30 to

40% of the iron in pork, liver and fish and 50 to 60% of iron in beef, lamb and chicken are in the form of heme (Cook and Monsen, 1976).

Nonheme iron consists of iron from foods such as vegetables, grains, fruits and eggs. Nonheme iron is also found in meats, poultry, fish and soluble iron supplements (Monsen et al., 1978; Schricker et al., 1982). Nonheme iron absorption is affected by the iron status of the individual (Olszon et al., 1978; Buchowski et al., 1989) and also by various dietary factors (Hallberg and Bjorn-Rasmussen, 1972; Cook and Monsen, 1976; Cook, 1983). Approximately 20% of nonheme iron is absorbed in the presence of enhancers (Monsen et al., 1978). Absorption of nonheme iron is significantly enhanced by ascorbic acid and animal tissue. Factors that inhibit the assimilation of food iron are tea, coffee, bran, protein and fiber (Cook, 1983). Although the percent absorption of nonheme iron is much lower than that of heme iron, a normal diet is higher in nonheme iron than in heme iron. Thus, the major contribution of available iron is made by nonheme iron (Monsen et al., 1978).

The above discussion presents the importance of heme, nonheme and total iron in nutrition. Considering the fact that iron deficiency is a relatively common problem throughout the world, especially in the underdeveloped and developing countries (Skikne, 1988), there is a need to assess and improve the levels of iron intake in the populations at risk. For this, an accurate estimate of the levels of heme, nonheme and total iron in foods is essential. An important goal of researchers in this field should be to improve the analytical methods used to develop data on food composition.

Validated and accurate methods should be used to analyze foods for heme, nonheme and total iron content. Validity of a method can be determined by measuring its accuracy, specificity and precision. Accuracy reflects the proximity of the replicate mean to the true value. Specificity is defined as the capacity of a procedure to produce a measurable response solely due to the presence of the analyte. Precision reflects the variation among replicates or among trials.

OBJECTIVES

The overall objective of this study is to determine the validity, i.e., accuracy, specificity and precision of different methods used to analyze heme, nonheme and total iron content of meats. The specific objectives of this study are:

1. To test the validity of heme and total iron methods by determining heme and total iron recovery from spikes of hemoglobin in meats.
2. To test the validity of heme iron, nonheme iron and total iron methods by determining if heme + nonheme = total iron in meats after conversion of varying amounts of heme to nonheme through baking.
3. To test the validity of heme and nonheme iron methods by determining the efficacy of the methods to detect varying concentrations of the analyte in a mixture of low and high heme iron food sources and by establishing the correlation coefficients between beef liver concentration and increase in heme iron content of beef liver/catfish mixtures for each of the methods.

LITERATURE REVIEW

To generate the needed level of complete, accurate and quantitative data on content and variation of "trace" minerals in foods, it is necessary to have adequate methodology for routine analysis of a sizable number of samples (Wolf, 1982). Errors introduced in trace metal analysis are manifold. These include errors introduced during sample collection, environmental contamination and inherent errors of instrumentation (Narayanan and Lin, 1985). Accurate analysis becomes more difficult when dealing with foods because of the chemically complex nature of food matrices. Many foods contain compounds which cause some assays to give results which are too high or too low (Stewart, 1989). Routine analytical procedures should be assessed based on meeting several criteria such as specificity, precision, sensitivity, critical evaluation, quality control and suitability for automation (Wolf, 1982). In validating analytical methodology, spike recovery techniques are necessary but not sufficient proof of accuracy; a basic requirement is the analysis of known, certified, primary standards (Wolf, 1982). There are many ways to validate data, including the use of standard reference materials, standard laboratories and internal standards. The choice of the data validation procedure, though, depends on the laboratory, the food samples, the component being measured (Stewart, 1989) and the availability of standard reference materials.

Quantitative determinations of heme, nonheme and total iron are often performed by workers in various fields, and considerable effort has been spent in establishing satisfactory methods. The following is a review of the methods that have been developed for the determination of heme, nonheme and total iron in biological materials.

Methods for the Determination of Heme Iron

There is a fundamental principle on which most quantitative measurements of heme iron are based. It is the extraction of heme pigments into solution followed by the spectrophotometric measurement of the derivatives. Hornsey (1956) employed acidified 80% acetone to extract the heme pigments and convert them to acid hematin. Earlier workers used water for extraction despite the finding of Whipple (1926) that water did not completely extract myoglobin from muscle (cited in Warriss, 1979). Other workers used buffers that ranged in pH from 4.5 to 7.0; one of the principal factors determining choice appearing to be ease of clarification of the final extract. Alkaline phosphate buffers were found to fully extract the pigment, but the solutions were difficult to clear. In 1950, Lowry (cited in Warriss, 1979) circumvented this problem by using a slightly acid buffer. Bowen and Eads (1949) showed that considerably lower pH buffers had the principal advantage of keeping the extracts clear (cited in Warriss, 1979). Warriss (1979) developed a method for extracting heme pigments from fresh meats using 80% acetone and 0.04 M phosphate buffer at pH 6.8.

Numerous experiments involving isolation of hemin have been conducted. Labbe and Nishida (1957) found that the hemin isolation methods have certain limitations and disadvantages such as the interference of excess protein in hemin crystallization, uncertain yields with small scale preparations, and time-consuming procedures when used for a large number of samples. Labbe and Nishida (1957) described a procedure for hemin isolation in which strontium chloride-acetone mixture is used as the extraction solvent.

Colorimetric determination of iron in natural products by means of o-phenanthroline color reagent has been described by several authors. The determination of iron specifically in hemin by such methods was first reported by Drabkin in 1941 (cited in Adler and George, 1965). Adler and George (1965) modified the Drabkin procedure to provide a somewhat more rapid and convenient determination. Cameron (1965) extended this method to apply to naturally occurring hemoproteins, hemoglobin and myoglobin. This

required digestion to destroy the protein, and modification of the assay conditions to prevent destruction of the ferrous o-phenanthroline complex formed.

Crosby et al. (1954) established a hemoglobin reference facility for the clinical laboratories of the armed services for the purpose of providing accurately measured, stable solutions of hemoglobin to be used as standards. This cyanmethemoglobin method for determination of hemoglobin concentration uses Drabkin's solution and is routinely used (Jansuittivechakul et al., 1986; Buchowski et al., 1989; Allred et al., 1990; Kalpalathika et al., 1991b).

Heme iron plays an important role in determining the color of meat. This is of particular interest to the meat producer because the color of the meat affects its marketability. Studies have been done to understand the nature of hematin complexes present in meats. Ledward (1974), studying the nature of the hematin-protein bonding in cooked meat, found that in cooked meat the hemoproteins are mainly di-imadazole complexes, the imadazole residues being supplied by the histidine groups of the bound protein. Ahn and Maurer (1990) studied the heme-complex-forming reactions of myoglobin, hemoglobin and cytochrome c with various ligands - pyridine, nicotinamide, albumin, histidine, cysteine and methionine. Among the ligands studied, pyridine and nicotinamide formed heme-complexes most effectively with all three pigments.

In recent years, considerable research has been done and different reagents have been found to be capable of extracting heme compounds. Some of the commonly used reagents are acetone, hydrochloric acid, strontium chloride, phosphate buffers and hydrogen peroxide for the decomposition of hemin compounds. The Hornsey technique of extracting heme with 80% acidified acetone is the most commonly used method for the determination of heme iron in foods (Jansuittivechakul et al., 1986; Buchowski et al., 1988 & 1989; Warriss et al., 1990; Kalpalathika et al., 1991a) because it is a fairly simple, and rapid technique. It is one of the methods tested for validity in this study.

Methods for the Determination of Nonheme Iron

The nonheme iron content of tissues was originally extracted in 1928 with 5 N HCl and determined with thiocyanate (Foy et al., 1967). The development of better chromogens led to the development of new methods. Tompsett (1934, 1935), studying the complexes of iron with biological materials, found that ferric iron is liberated from its complexes by reducing agents such as thiolacetic acid and sodium hydrosulphite. Ferric iron is also liberated from these complexes by sodium pyrophosphate due to the formation of un-ionized ferric pyrophosphate. Ferrous iron formed no such complexes. The liberated iron was determined with thioglycolic acid. Hill et al. (1930) extracted nonheme iron with reducing agents and bipyridine and quantified it with α,α'-bipyridine reagent (cited in Bruckmann and Zondek, 1940). Bruckmann and Zondek (1940) modified the method originally proposed by Tompsett (1935) and showed that trichloroacetic acid-pyrophosphate solution markedly improved the extraction of nonheme iron from tissues. Foy et al. (1967) described an improved method for measuring the nonheme iron content of tissue specimens using TCA-sodium pyrophosphate mixture as the extraction solvent and α,α'-bipyridine as the color reagent. A method for the determination of nonheme iron in bone marrow was described by Kerr (1957) in which sodium sulphite and dipyridyl reagent were used as extracting reagents, and α,α'-dipyridyl was used as the color reagent.

Later, bathophenanthroline color reagent has been used in the determination of nonheme iron. Examining the nonheme iron method proposed by Igene et al. (1979), Rhee and Ziprin (1987) found that it might underestimate the nonheme iron content of muscle tissues as a result of insufficient extraction/recovery of nonheme iron proteins (ferritin, transferrin and others) from the muscle. The Schricker et al. (1982) method for determining nonheme iron was developed from the Torrance and Bothwell (1968) method that was used to clinically determine iron status of liver from biopsies. Rhee and Ziprin (1987) suggest that the method developed by Schricker and coworkers is capable of overestimating the nonheme iron content of red meats because of pigment effects. They

developed a more accurate nonheme iron assay procedure for red meats by modifying the Schricker method through minimization of pigment effects. One of the methods currently used routinely in laboratories is the Schricker method (Chen et al., 1984; Buchowski et al., 1988 & 1989) with the modifications suggested by Rhee and Ziprin (Bowers et al., 1989; King et al., 1990). The validity of two nonheme iron methods, the modified Schricker method as modified by Rhee and Ziprin and the sodium pyrophosphate extraction method, are tested in this study.

Methods for the Determination of Total Iron

For the determination of total iron in biological material, atomic absorption spectroscopy (AAS) is relatively simple and has widespread use (Lee and Clydesdale, 1979). Caraway (1963) described a method for the determination of serum iron and iron binding capacity using the color reagent TPTZ (2,4,6-tripyridyl-s-triazine). Serum iron determinations were conducted by Sharma et al. (1969) using dimethyglyoxime and Morris (1952) described a method for the determination of iron in water in the presence of heavy metals using tripyridyl solution. The development of automated analytical systems for the determination of serum iron and iron binding capacity using iron chelating ligands was reviewed by Klein (1971). Vuori et al. (1963) modified a procedure originally proposed by Trinder in the 1950's in which bathophenanthroline color reagent was used.

Lee and Clydesdale (1979) developed a method for the simultaneous quantification of the various forms of iron added or endogenous to foods. The total, elemental and soluble iron were determined with minimal pre-treatment by atomic absorption spectrophotometry. The iron valences and complexed iron were measured spectrophotometrically using bathophenanthroline color reagent. Torrance and Bothwell (1968) proposed a simple technique for measuring storage iron concentrations in formalinised liver samples using bathophenanthroline color reagent. Bathophenanthroline color reagent has been popular for the quantification of total iron since it reacts exclusively

with ferrous iron to form a deep-red colored complex. Bathophenanthroline has been shown to be highly reliable for the determination of ferrous iron in waters and has been shown to accurately determine ferrous iron in the presence of large amounts of ferric iron in metallurgy. Bathophenanthroline is extractable in organic solvents and thus the ferrous-batho chelate may be separated from most interfering substances. Phosphates do not interfere with the ferrous-batho complex. Bathophenanthroline reagent follows Beer's law and is very stable (cited in Lee and Clydesdale, 1979). Wickramasinghe (1974) successfully used bathophenanthroline in the investigation of acid-labile protein-iron in iron-sulphur proteins and ferroflavoproteins.

Stookey (1970) and Carter (1971) described methods for spectrophotometric determination of total iron using ferrozine. The absorption spectrum of the ferrous-ferrozine complex shows a sharp peak with maximal absorbance in the visible range at 562 nm. Ferrozine exhibits a molar extinction coefficient of 28,000 and is stable at room temperature for at least 30 min (Carter, 1971). This compound reacts with divalent iron to form a stable magenta colored complex which is very soluble in water (Stookey, 1970). Gibbs (1976) found, after careful rechecking over a tenfold range of iron concentrations, a value of 28,600 as the molar absorptivity of ferrozine. The kinetics of complexation of iron (II) with ferrozine were compared to that of 1,10-phenanthroline and 2,2'-bipyridine (two other commonly used reagents). It was seen that in the pH range of analytical use, the complex formation with ferrozine could be considered as favored and fast. The advantage of ferrozine is that it works at higher rates than the other two reagents at lower pH values (Thompsen and Mottola, 1984). Since its introduction by Stookey in 1970, ferrozine iron reagent has been applied to determination of iron in a wide range of situations. Its advantages include its water solubility, low cost and sensitivity (Gibbs, 1976).

Derman et al. (1989) compared the chromogenic substrates ferrozine and ferene with bathophenanthroline disulphonic acid for the measurement of iron concentrations in aqueous and serum samples in an assay based on that of the Iron Panel of the International

Committee for Standardization in Hematology. Ferrozine and ferene were seen to be more sensitive than bathophenanthroline. Interference by copper was minimal with all three chromogens when thioglycolic acid was used as the reducing agent, but when ascorbic acid was used, significant positive interference by copper was seen with ferrozine and ferene. Duffy and Gaudin (1977) conducted experiments which demonstrated that copper will react with ferrozine to give a copper (I)-ferrozine complex with 10% of the molar absorptivity given by the ferrous (II)-ferrozine complex. Thiourea was seen to prevent more than ninety percent of the interference due to copper by forming a copper (I)-thiourea complex which effectively masks the copper against ferrozine.

Salinas et al. (1986) synthesized and studied a new reagent, 5,5-dimethyl-1,2,3-cyclohexanetrione 1,2-dioxime 3-thiosemicarbazone (DCDT); a simple, rapid, selective and sensitive method for the spectrophotometric determination of iron in wines, minerals and foods (spinach and lentils) using this reagent was described. Kawasaki et al. (1990) developed a simple, rapid, selective and sensitive method for the determination of iron (III) ion by ion chromatography coupled with electrochemical detection. The method was seen to reduce the interferences of iron (II) ions and enabled more than 5 pmol of iron (III) to be determined with an injection volume of 10 µl. The method was applied to the determination of ferroxidase activity of ceruloplasmin with good reproducibility.

Atomic absorption spectrophotometry

For mineral determination in foods, atomic absorption spectrophotometry (AAS) is most widely used (Harnley and Wolf, 1984). Attributes are instrument operation simplicity, excellent element specificity, capability of measuring about 70 elements, and moderate cost of basic instrumentation (Alvarez, 1984).

Atomic absorption spectrophotometry makes use of the fact that neutral or ground state atoms of an element can absorb light and electromagnetic radiation over a series of very narrow, sharply defined wavelengths. The sample, in solution, is aspirated as a fine

mist into a flame where it is converted to an atomic vapor. Most of the atoms remain in ground state and are therefore capable of absorbing radiation of a suitable wavelength. This discrete radiation is supplied by a hollow cathode lamp, which is a sharp line source consisting of a cathode containing the element to be determined along with a tungsten anode. Since, generally, only the test element can absorb this radiation, the method becomes very specific in addition to being sensitive (Atomic Absorption Methods Manual, 1975).

As in colorimetric determinations, the major disadvantage of AAS is that the sample has to be in solution and so has to be treated prior to analysis. Interferences of other elements with the analysis of iron has also been reported. Iron or copper decreases AAS detection of zinc in mineral solution but not in protein solutions; calcium and magnesium have a negative effect on the atomic absorption of manganese (Favier et al., 1985). Phosphorus interferes with the detection of iron, manganese, magnesium and cadmium by AAS. To minimize these interferences, Ln or Sr are commonly put in the sample solutions. Also common is the use of NBS reference materials alongside the sample to check for accuracy of analysis during each run. Other drawbacks of AAS have historically been its limited calibration range and its inability to analyze more than one element at one time (Harnley and Wolf, 1984).

Davies et al. (1972) compared the colorimetric and atomic absorption procedures in the determination of iron in low-iron diets. They found that colorimetric (sulphonated bathophenanthroline) and atomic absorption methods gave mean values which were not significantly different; however, values obtained with atomic absorption were significantly more variable. Copper, phosphate, perchlorate and sulfate did not interfere with color development. Nielson et al. (1988) validated the X-ray fluorescence (XRF) method using NBS reference materials. The XRF and AAS measurements compared agreeably for 96 samples from different sources of 21 foods. Simultaneous multielement atomic absorption continuum source spectrometer (SIMAAC) with flame atomization is now being used

routinely at the USDA for multielement determinations in foods, agricultural and biological materials (Harnley and Wolf, 1985).

The chromogenic substrate that is most commonly used today for colorimetric determinations of iron in food samples is ferrozine (Buchowski et al., 1988 & 1989; Zhang and Mahoney, 1990 & 1991; Kalpalathika et al., 1991b). The use of atomic absorption spectroscopy for total iron determinations is also common (Schricker et al., 1982; Jansuittivechakul et al., 1986; Nielson et al., 1988; Bowers et al., 1989; Allred et al., 1990; King et al., 1990; Marti et al., 1990; Falandysz, 1991). The ferrozine method and AAS are the two total iron methods that will be tested for validity in this study.

Preparation of the Sample - Ashing Techniques

The interest in trace metals as elements for normal biological development or as potential sources of danger to health has resulted in many reports dealing with methods for their determination in biological materials (Yang et al., 1990). Dry and wet ashing are two commonly used methods for the preparation of biological materials for trace element analysis by atomic absorption spectrophotometry (Clegg et al., 1981b), inductively coupled plasma atomic emission spectrometry and sometimes voltammetric methods. Baker and Smith (1974) studied various ashing procedures for preparation of plant tissue for AAS analysis. They found that dry ashing decreased the apparent iron levels and, therefore, they proposed wet ashing using nitric, perchloric, or sulfuric acids (cited in Lee and Clydesdale, 1979). Friel and Ngyuen (1986) compared dry and wet ashing techniques in analyses of iron in hair. They found that iron was lost during dry-ashing of hair, and that wet digestion with nitric acid, rather than $HClO_4$:HNO_3, is acceptable for the analysis of iron in hair. They recommend dry ashing for the analysis of zinc, copper and manganese, and wet ashing with nitric acid for assays of iron.

Clegg et al. (1981a) evaluated the dry ashing efficiency by comparing iron, copper, zinc and manganese concentrations of the samples with NBS-certified values. Highest

recoveries were obtained by dry ashing in silica glass (Vycor) crucibles. Dissolving the resultant ash in either hydrochloric or nitric acids did not significantly alter the results. A comparison between dry and wet ashing showed the latter method was superior for the preparation of biological tissues for analysis of iron, copper, zinc and manganese. The same authors examined several common methods of wet ashing using NBS bovine liver to determine which acids, acid combinations or bases should be used as digesting agents for accurate and precise measurement of iron, copper, zinc and manganese. They found nitric acid to be superior to other acids (HCl and H_2SO_4), acid combinations or bases for the digestion of liver samples. Various other acid combinations such as nitric-perchloric, nitric-hydrochloric, nitric-sulfuric and nitric-hydrogen peroxide were tested and found to give good results. The nitric-perchloric mixture was superior to nitric acid alone in the breakdown and dispersion of fat in the sample. However, the greater potential for trace element contamination and for laboratory accidents makes this acid combination less desirable than nitric acid alone. They concluded that nitric acid digestion is a rapid, accurate and relatively safe technique for the determination of iron, copper, zinc and manganese in a variety of animal tissues (Clegg et al., 1981a).

Yang et al. (1990) described a method combining radiotracer techniques with electrophoresis which provides a unique possibility to study the relative effectiveness of the decomposition process of biological samples. A one-cycle reflux digestion showed good recoveries for cobalt and selenium in liver samples. Among the liver samples tested, the samples containing zinc were found to be the most difficult to digest by wet oxidation; a three-cycle digestion process seemed to ensure complete release of zinc from the biological matrix. Hill et al. (1986) developed a procedure for digestion of biological materials for mineral analyses using a combination of wet and dry ashing. Using this procedure, the authors found no loss of the seven biologically important minerals that were tested, i.e., Cu, Mn, Fe, Zn, Cr, Ca and Mg. Other advantages of this procedure are (1) very little operator time is required and (2) only small amounts of reagents are needed lowering the

chance of contamination and allowing for more accurate analysis at low concentrations of analyte in samples.

The AOAC (1990) recommends wet ashing with nitric acid and addition of hydrogen peroxide to complete the digestion process; the resultant ash to be dissolved in diluted solution of HCl. This is the procedure followed in this study to prepare the samples for total iron analysis using ferrozine color reagent and atomic absorption spectrophotometry.

In recent years, the original methods have undergone modifications. They have been modified to suit individual and specific needs. For example, the Schricker et al. (1982) method for the determination of nonheme iron was modified by Buchowski et al. (1988) for their experiments. They used ferrozine as the color reagent in place of bathophenanthroline disulfonate. Investigators have modified the methods to overcome certain limitations but little has been done to know the methods that provide the best results while imposing minimum constraints.

In this study some well-established methods used to determine heme, nonheme, and total iron content in meats will be compared, and their validities will be determined.

One heme, two nonheme and two total iron methods were tested in this study. Criteria upon which the methods were chosen for this study are:

1. The methods should be currently used by many workers.
2. The methods should differ significantly from each other with regard to the reagents used or the manner in which the desired compounds are extracted.

MATERIALS AND METHODS

Experimental Design

The objective of determining the validity of some heme, nonheme and total iron methods was achieved in three experimental procedures.

In experiment one, the accuracy, specificity and precision of heme iron and total iron methods were determined by spike recoveries. Hemoglobin was used as the spike on a rich source of iron - raw beef, and a poorer source - raw chicken. Since 1 g of hemoglobin contains 3.35 mg iron (Mahoney and Hendricks, 1982), this figure was used as the reference for determining the accuracy and specificity of total iron methods. Hemoglobin spike recoveries (%) allowed further establishment of the validity of the heme and total iron methods.

In experiment two, the validity of heme, nonheme and total iron methods were determined by facilitating the conversion of varying amounts of heme iron to nonheme iron and analyzing for heme, nonheme and total iron. It has been demonstrated that cooking leads to destruction of heme in foods (Igene et al., 1979; Schricker and Miller, 1983; Chen et al., 1984; Buchowski et al., 1988). A common source of iron in a typical American diet, ground beef patties, was baked to different degrees of doneness - rare, medium and well-done. The decrease in heme iron and an equal increase in nonheme iron as the meat is baked to a higher degree, and the total iron at each point identical to the sum of heme iron and nonheme iron at that point, was used as the basis for determining the accuracy, specificity, and precision of heme, nonheme and total iron methods.

In experiment three, the specificity and precision of heme iron and nonheme iron methods were determined by analyzing mixtures of beef liver (rich in iron) and catfish (poor source of iron) mixed in different proportions. As the concentration of beef liver in the mixture decreases, the heme and nonheme iron content also decreases and vice versa. The linearity (correlation coefficients) obtained by plotting heme or nonheme iron

concentrations against the beef liver proportion in the mixture reveals the completeness of the extraction methods.

To further establish the accuracy, specificity and precision of the total iron methods, NIST (National Institute of Science and Technology) certified reference materials were analyzed for total iron.

Food Collection and Preparation

Experiment one: Determining the validity of heme and total iron methods using spikes of hemoglobin in raw beef and raw chicken samples.

Raw, lean, ground beef patties, bought from Dept. of Nutrition & Food Sciences meat laboratory, and raw chicken thighs, bought from Macey's, Logan, were used. The beef patties were used as such, and the chicken thighs were cut to fine pieces using a plastic board and stainless steel meat knife. For spiking purposes, lyophilized hemoglobin (from Sigma Chemical Co.) was used.

Experiment two: Determining the validity of heme iron, nonheme iron and total iron methods using lean ground beef patties baked rare, medium and well-done.

Lean ground beef patties were bought from the Dept. of Nutrition & Food Sciences meat laboratory. The patties were placed in a glass petri dish and baked in a conventional air oven at 105°C to internal temperatures of 60°C (rare-done), 74°C (medium-done) and 80°C (well-done). The internal temperatures were checked using a meat thermometer. The baked patties were then analyzed for heme, nonheme and total iron.

Experiment three: Determining the validity of heme and nonheme iron methods using beef liver and catfish mixed in varying proportions.

Raw, pureed liver, purchased from the Dept. of Nutrition and Food Sciences meat laboratory, and Smith's Food & Drug Store, Logan, and raw catfish steak, purchased from Smith's Food & Drug Store, Logan, were used. The catfish steak was chopped into fine pieces using a plastic board and stainless steel meat knife. These were weighed and mixed

to the proportions required separately for each individual replicate. The mixture was then analyzed for heme and nonheme iron.

Foods were stored frozen in plastic Ziploc® bags until ready for analysis. In experiments one and two, the analyses were run on 5 samples, each sample analyzed in triplicate. In experiment three, 3 samples each were analyzed in triplicate.

Analytical Methods

Heme iron determination - Hornsey method

Hornsey (1956) described a method in which he employed 80% acidified acetone to convert the heme pigments to acid hematin. Triplicate 10 g aliquots of the ground sample were weighed (±0.0001 g) and placed in plastic tubes. Out of the 40 ml of 80% acidified acetone to be added to each tube, a few ml were added, and the samples were mixed to a smooth paste using the polytron. The remaining solution was added to the respective tubes, mixed and filtered after 1 h. Absorption of the filtrates was measured at a wavelength of 640 nm, with 80% acetone-water solution as a blank. Heme iron concentration was calculated by the equation:

$$\mu g \text{ heme iron/g sample} = \frac{\text{OD at 640 nm X 680 (extinction coefficient)}}{\text{wgt of sample (g)}}$$

In the case of hemoglobin spiked samples, 0.01 g and 0.02 g of hemoglobin was weighed (±0.0001 g) and added to the tubes before the addition of the extraction solvent. Spike recoveries were calculated assuming that Hb contains 3.35 mg Fe/g Hb. The following equation was used:

$$\text{Spike recovery (\%)} = \frac{\text{Fe calculated (}\mu g/g\text{)}}{\text{Fe true value (}\mu g/g\text{)}} \quad X \quad 100$$

Nonheme iron determination

Modified Schricker method. A modification of the nonheme iron method of Schricker et al. (1982), developed by Rhee and Ziprin (1987) was followed. Triplicate 5 g aliquots of the ground sample were weighed (±0.0001 g) and placed in glass tubes. The samples were mixed with 0.2 ml $NaNO_2$ reagent and 15 ml of the acid mixture was added to each tube and incubated in a water bath-shaker at 65°C for 20 h. After cooling, 5 ml of color reagent was added to 1 ml of acidic liquid above the meat residue, and centrifuged at 3500 rpm for 10 minutes. Absorbance of the supernatant was read at 540 nm against the reagent blank (1 ml acid mixture + 5 ml color reagent).

To obtain the liquid phase blank value, 1 ml of the acidic liquid above the meat residue was mixed with 5 ml of saturated sodium acetate/water mixture (mixed in a ratio 20:21). The contents were centrifuged at 3500 rpm for 10 minutes and the absorbance was read at 540 nm against the reagent blank (1 ml acid mixture + 5 ml of the reagent used above). This value was subtracted from the reading obtained using the color reagent. The iron concentration was read off the working curve obtained using standards of 0.5, 1, 2, 3, 4, 5, and 10 ppm made from a 1000 ppm iron stock solution. The nonheme iron concentration of the sample was obtained by the following equation:

μg nonheme iron/g sample = Fe conc. of the incubated liquid phase (μg/ml) X (15 ml + 0.2 ml + moisture content (ml) of sample) ÷ wgt of sample (g)

Sodium pyrophosphate method. The sodium pyrophosphate extraction methods described by Bruckmann and Zondek (1940) and Foy et al. (1967) were used, and no color was observed. The extraction time was increased while decreasing the extraction temperature. The procedure followed was: 10 ml of 10% trichloroacetic acid (w/v) and 5 ml of saturated sodium pyrophosphate solution were added to triplicate 1 g (weighed to ±0.0001 g) aliquots of the ground sample placed in screw-cap glass culture tubes. The tubes were

tightly capped and left in a water bath at 55-60°C for 16-18 h. The solutions were cooled and filtered. One ml of the clear, colorless filtrates was taken in a test tube and 1 ml reducing agent was added and left for 30 min. Bathophenanthroline color reagent (prepared as described by Rhee and Ziprin, 1987) was added to the tubes, and the solutions were left for 30 min for the color to develop before centrifuging at 3500 rpm for 10 min. The absorbance was read at 540 nm against a blank in which the sample solution was replaced with deionized water. The iron concentration was read off the working curve obtained using standards of 0.5, 1, 2, 3, 4, 5, and 10 ppm made from a 1000 ppm iron stock solution. The nonheme iron concentration of the sample was obtained by the following equation:

μg nonheme iron/g sample = Fe conc. of the filtrate X (15 ml + moisture content (ml) of sample) ÷ wgt of sample (g)

Total iron determination

Wet ashing. All samples analyzed for total iron were wet ashed. Wet ashing was done as follows: triplicate 1 g aliquots of the ground samples were weighed (±0.0001 g) and placed in 25 ml Erlenmeyer flasks with 10 ml concentrated HNO_3 and 2-3 glass beads. The flasks were placed on a hot plate at low heat for 1-2 days and then allowed to go to dryness. If white ash was not obtained, 5 ml of 30% H_2O_2 were added, to each flask and a white ash was obtained in 24 h. After cooling the flasks to room temperature, 2 ml of concentrated HCl were added, and the solution was made to appropriate volume with deionized water. Each of the ash solutions was analyzed for total iron by the ferrozine and AAS methods.

In case of hemoglobin spiked samples, 0.01 g and 0.02 g of hemoglobin was weighed (±0.0001 g) and added to previously weighed meat samples in the flasks. Wet ashing iron analysis procedures were carried out the same way as for unspiked samples.

Ferrozine method. Triplicate 0.5 ml aliquots of the prepared ash solution were pipetted into a test tube and a drop of ortho-nitrophenol indicator was added to each tube. The pH of the solution in each tube was adjusted to approximately 5.5 using 6 N ammonium hydroxide and 0.5 ml of reducing agent, 10% hydroxylamine hydrochloride (w/v), was added. The mixture was allowed to stand at room temperature for 15 minutes. To each tube, 1 ml of buffer solution, 10% ammonium acetate (w/v), was added, followed by the addition of 1 ml of 1 mM ferrozine color reagent. The solution was brought to 5 ml volume with deionized water. The absorbance of the magenta colored complex was measured spectrophotometrically 45 minutes later at 562 nm against a blank in which the sample solution was replaced by water. A standard curve was prepared using standards of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 10.0 ppm made from a 1000 ppm iron stock solution. The iron content of the filtrate was read off the standard working curve. The total iron in the sample was calculated by the equation:

$$\mu g \text{ total iron/g sample} = \frac{\text{Fe conc of the filtrate X volume of ash solution (ml)}}{\text{wgt of sample (g)}}$$

Spike recoveries were calculated assuming that Hb contains 3.35 mg Fe/g Hb. The following equation was used:

$$\text{Spike recovery (\%)} = \frac{\text{Fe calculated } (\mu g/g)}{\text{Fe true value } (\mu g/g)} \times 100$$

Atomic absorption analysis. The prepared sample solutions were analyzed for total iron in triplicates by AAS (Instrumentation Laboratories Model 457 atomic-absorption spectrophotometer). Iron standard curves were obtained using standards of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 10.0 ppm made from a 1000 ppm iron stock solution. The iron concentration of the solution was read off the standard curve. The total iron in the sample was calculated by the equation:

$$\mu g \text{ total iron/g sample} = \frac{\text{Fe conc of the filtrate X volume of ash solution (ml)}}{\text{wgt of sample (g)}}$$

Spike recoveries were calculated assuming that Hb contains 3.35 mg Fe/g Hb. The following equation was used:

$$\text{Spike recovery (\%)} = \frac{\text{Fe calculated (}\mu g/g\text{)}}{\text{Fe true value (}\mu g/g\text{)}} \text{ X } 100$$

Moisture determination

Moisture determinations of raw and baked lean ground beef patties, raw chicken thighs, raw liver (pureed) and raw catfish steak were done by oven-drying 1-2 g (initial weight) samples at 100-102°C for 16-18 h and cooling the samples in a desiccator. The samples were weighed (final weight), and weight loss was reported as moisture (AOAC, 1990). Moisture content of the sample was determined by the equation:

$$\text{Moisture (\%) in sample} = \frac{\text{initial wgt (g)} - \text{final wgt (g)}}{\text{initial wgt (g)}} \text{ X } 100$$

Statistical Procedures

In experiment one, the values obtained by the ferrozine method were compared to determinations by AAS for raw beef, raw chicken and hemoglobin samples. Means, standard deviations and coefficients of variation were calculated. One-way ANOVA was performed at the 95% confidence level to determine significance of difference between means obtained by the two methods.

F tests were also performed at the 95% confidence level to determine significance of difference among mean percentage spike recoveries obtained by the Hornsey method, ferrozine method and AAS for 0.01 g and 0.02 g hemoglobin spiked raw beef samples. The same tests were done in the case of 0.01 g and 0.02 g hemoglobin spiked raw chicken samples. Fischer's LSD was used when F was statistically significant ($p<.05$).

The mean percentage spike recoveries in spiked raw beef and spiked raw chicken samples obtained by the Hornsey method, ferrozine method and AAS were tested for possible significant difference from 100% recovery at the 99% confidence level using student's t test for small sample inferences about a population mean. Means, standard deviations and coefficients of variation were calculated.

In experiment two, nonheme iron determinations were made by the modified Schricker and sodium pyrophosphate methods and total iron determinations were made by the ferrozine and AAS methods in rare, medium and well-done ground beef. Means, standard deviations and coefficients of variation were calculated. A two-way ANOVA was performed at the 95% confidence interval to determine significance of difference between means obtained by the two nonheme methods (or the two total iron methods) and among mean nonheme iron values (or mean total iron values) of beef samples baked to the three degrees of doneness. Fischer's LSD was used when F was statistically significant ($p<.05$).

The total iron values obtained by the summation of heme and nonheme iron values were compared to the total iron values obtained by the total iron methods. The mean difference between heme+nonheme and total iron values and the standard deviation of the mean difference were calculated. Student's t test was performed at the 95% confidence level to determine significant differences between means.

In experiment three, the nonheme iron determinations in beef liver/cat fish mixtures were made by modified Schricker and sodium pyrophosphate methods. Means, standard deviations and coefficients of variation were calculated. A two-way ANOVA was performed at the 95% confidence level to determine significance of difference between means obtained by the two methods and among means of nonheme iron content of the five liver/fish mixtures under study. Fischer's LSD was used when necessary. The heme and nonheme iron concentrations were plotted against the liver concentrations in the mixture,

and correlation coefficients between beef liver concentration and heme or nonheme iron content were obtained.

Coefficients of variation (CV) were calculated by dividing the sample standard deviation by the mean and then multiplying by 100 to give a percent value (CV=SSD/mean x 100). The overall coefficient of variation for a method was calculated from the individual CVs for all experiments by the following equation:

$$\text{Overall CV} = (CV_1 \times n_1) + (CV_2 \times n_2) \div (n_1+n_1)$$

RESULTS

Experiment One

In experiment one, the accuracy, specificity and precision of heme and total iron methods was tested using spikes of hemoglobin in raw beef and raw chicken samples. Also, since hemoglobin contains 3.35 mg of iron per gm (Mahoney and Hendricks, 1982), this figure was used as the reference for determining the accuracy and specificity of the total iron methods.

The data presented in Table 1 shows that raw ground beef and raw chicken thighs have a mean heme iron value of 25.6 ± 1.2 μg/g and 5.7 ± 0.5 μg/g, respectively, on a fresh weight basis. The spike recoveries as heme iron ranged from 84.4% to 131.1% in raw beef samples and 79.7% to 106.2% in raw chicken samples. Student's t test for small sample inferences about a population mean indicates that the mean percentage spike recoveries were not significantly different ($p>.01$) from 100%. Also, a one-way ANOVA showed that there were no significant differences ($p>.05$) between spike recoveries for the 0.01 g and 0.02 g hemoglobin spikes indicating that the heme iron concentration in the sample did not affect the spike recoveries by the Hornsey method. The Hornsey method was able to completely extract up to 323 μg of heme iron in a sample, the highest amount tested in this study.

Determining the total iron content of hemoglobin, the ferrozine method gave a value of 3.35 ± 0.09 mg/g (n=15) and the AAS method gave a value of 3.25 ± 0.23 mg/g (n=15). The two values were not significantly different ($p>.05$). The mean total iron values in raw ground beef samples as determined by the ferrozine and the AAS methods were 36.7 ± 1.2 μg/g and 31.9 ± 2.3 μg/g, respectively, on a fresh weight basis. The ferrozine and AAS values were found significantly different ($p<.05$) by one-way ANOVA at the 95% confidence level. Similarly, the total iron content of raw chicken thighs as determined by ferrozine, 11.6 ± 1.2 μg/g, was higher ($p<.05$) than that determined by

AAS, 8.7 ± 0.4 μg/g (Table 2). It was found that 69.8% of the total iron in raw ground beef and 49.1% in raw chicken thighs is in the heme form.

Table 1 - Summary of heme iron values of hemoglobin spiked and unspiked raw beef and raw chicken thigh samples determined by the Hornsey method

Sample	% moisture	unspiked(μg/g fresh weight)	Spike recoveries (%) of 01g Hb	Spike recoveries (%) of .02g Hb	diff[a]	F-test[b]
Beef[c]	56.5 ±1.0	25.6 ±1.2	95.6 ±13.8	101.1 ±9.6	5.5	NS
Chicken[c]	74.7 ±1.8	5.7 ±0.5	96.1 ±5.2	94.1 ±7.6	2.0	NS
student's t test[d]			NS	NS		

[a]Difference between the mean spike recoveries of .01g and .02g Hb spikes.
[b]One-way ANOVA performed at 95% confidence level; NS=no significant difference.
[c]Means and standard deviations of 5 spiked and unspiked samples. Each sample was analyzed in triplicate.
[d]Student's t-test performed at the 99% confidence level to test mean spike recoveries against H_0:μ=100%; NS=no significant difference.

Total iron spike recoveries from hemoglobin spikes in raw ground beef and raw chicken thighs are presented in Table 3. Total iron recoveries from hemoglobin spikes using the ferrozine method ranged from 90.0% to 103.7% in raw beef samples and 81.3% to 108.6% in raw chicken samples. For the AAS method, the values ranged from 77.0 to 95.3% in raw beef samples and 78.3 to 97.2% in raw chicken samples. Student's t test revealed that the ferrozine mean spike recoveries were not significantly different ($p>.01$) from 100%; however, the AAS mean spike recoveries were significantly less than 100% ($p<.01$). The AAS values for hemoglobin were very close to the reference value, 3.35 mg Fe/g. This may be due to the homogeneity of the sample; the presence of other minerals in food samples interferes with the ability of the atomic absorption spectrophotometer to quantitatively detect a given concentration of iron. Thus, the same accuracy was not seen when a heterogeneous product such as beef or chicken samples were used. A one-way

ANOVA revealed that there were no significant differences ($p>.05$) between mean percentage total iron recoveries obtained by either the ferrozine or AAS methods for the 0.01 g and 0.02 g hemoglobin spikes in raw beef and raw chicken samples indicating that the total iron concentrations of the samples did not affect the detection ability of the methods.

The individual coefficients of variation for each sample are given in the appendix.

Table 2 - Summary of ferrozine and atomic absorption spectrophotometry (AAS) values for total iron in hemoglobin (mg/g), raw beef and raw chicken thigh samples (µg/g fresh weight)

Sample	% moisture	Ferrozine[a]	AAS[b]	diff[c]	F-test[d]
Hemoglobin		3.35 ±0.09	3.25 ±0.23	1.0	NS
Beef	56.5 ±1.0	36.7 ±1.2	31.9 ±2.3	4.8	SD
Chicken	74.4 ±1.8	11.6 ±1.2	8.7 ±0.4	2.9	SD

[a]Ferrozine means and standard deviations
[b]AAS means and standard deviations
[c]Difference between ferrozine and AAS means
[d]One way ANOVA performed at the 95% confidence level; NS=no significant difference, SD=significantly different

Table 3 - Total iron spike recoveries (%) in hemoglobin spiked raw beef and raw chicken thigh samples determined by ferrozine and AAS methods

	Spike of 0.01g Hb		Spike of 0.02g Hb		
Method	Beef	Chicken	Beef	Chicken	t test[a]
Ferrozine[b]	98.2 ±2.9	95.7 ±7.8	98.2 ±2.9	99.3 ±3.2	NS
AAS[b]	85.0 ±3.5	86.6 ±3.9	85.6 ±6.0	85.7 ±4.4	SD

[a]Student's t test performed at the 99% confidence level to test mean spike recoveries against H_o:µ=100%; NS=no significant difference, SD=significantly different.

[b]Means and standard deviations of 5 beef and chicken samples. Each sample was analyzed in triplicate.

Experiment Two

The best way to test for complete extraction is to compare the method in question with an indirect one, in which the nonheme iron level is calculated from the difference between the total iron and heme iron levels (Bruckmann and Zondek, 1940). This principle is used in this experiment.

Ground beef was used as the sample because it is a high heme iron source and constitutes one of the major sources of iron in a typical American diet. One way of destroying varying amounts of heme iron is by baking the food to different degrees of doneness -- rare, medium and well-done.

Heme, nonheme and total iron concentrations obtained by the different methods presented in Table 4 shows that as ground beef was baked to higher internal temperatures, the heme iron content decreased slightly and nonheme iron content increased significantly ($p<.05$) while the total iron content remained fairly constant ($p>.05$). The heme iron content of well-done beef determined by the Hornsey method is seen to have increased even though the nonheme iron content determined by the two nonheme iron methods also increased. This may be due to the browning of the well-done beef patty that may have added to the color of the filtrate giving a higher absorbance value and thus a higher heme iron value.

Table 4 - Heme, nonheme and total iron content (μg/g wet weight) of baked, ground beef determined by various methods

		Heme[a]	Nonheme[b]		Total iron[c]	
Sample[d]	% moisture	Hornsey	M. Schricker	$Na_4P_2O_7$	Ferrozine	AAS
Rare	53.6 ±1.6	22.7 ±1.8	13.5 ±1.1	10.9 ±0.7	35.3 ±1.4	30.7 ±1.6
Medium	51.4 ±1.1	21.4 ±1.2	15.9 ±0.5	13.9 ±0.6	34.7 ±2.0	29.7 ±2.2
Well-done	49.5 ±0.7	23.9 ±1.7	17.2 ±1.1	16.7 ±0.8	35.2 ±2.3	27.6 ±1.3

[a]Heme iron values are the means and standard deviations of 5 determinations made by the Hornsey method. Each determination is a mean of triplicate analytical values.

[b]Nonheme iron values are the means and standard deviations of 5 determinations using modified Schricker and sodium pyrophosphate methods. Each determination is a mean of triplicate analytical values.

[c]Total iron values are the means and standard deviations of 5 determinations using the ferrozine and AAS methods. Each determination is a mean of triplicate analytical values.

[d]Samples were baked to an internal temperature of 60°C (rare), 74°C (medium) or 80°C (well-done).

Two-way ANOVA revealed no significant differences ($p>.05$) between the values obtained by the two nonheme iron methods, modified Schricker and sodium pyrophosphate extraction; but, there is significant difference ($p<.05$) in nonheme iron content of rare, medium and well-done ground beef patties. Two-way ANOVA, however, revealed significant differences ($p<.05$) between the values obtained by the two total iron methods, ferrozine and AAS; there is no significant difference ($p>.05$) in total iron content of rare, medium and well-done ground beef patties for either procedure (Table 4).

Table 5 - Comparison of the summation of Hornsey (heme) and modified Schricker (nonheme) iron determinations with ferrozine and AAS (total) iron determinations in baked ground beef patties (μg/g wet weight)

	Heme + Nonheme	Total Iron			
Sample[a]	Hornsey+Schricker[b]	Ferrozine[c]	diff[e]	AAS[d]	diff[e]
Rare	35.9 ±1.9	35.3 ±1.4	-0.45(NS) ±2.49	30.7 ±1.6	-5.04(SD) ±3.02
Medium	37.4 ±1.4	34.7 ±2.0	-2.44(NS) ±1.99	29.7 ±2.2	-7.71(SD) ±2.25
Well-done	41.1 ±1.9	35.2 ±2.3	-6.22(SD) ±2.26	27.6 ±1.3	-13.9(SD) ±2.11

[a]Samples were baked to an internal temperature of 60°C (rare), 74°C (medium) or 80°C (well-done).
[b]Means and standard deviations obtained by the summation of values of the Hornsey and modified Schricker methods (n=5, each sample was analyzed in triplicate).
[c]Ferrozine means and standard deviations of 5 samples; each sample was analyzed in triplicate.
[d]AAS means and standard deviations of 5 samples; each sample was analyzed in triplicate.
[e]Mean difference (between heme+nonheme and total) and standard deviation of the mean difference. Student's t test at the 95% confidence level; NS=no significant difference, SD=significantly different.

The heme and nonheme iron, when added, represents the total iron content of the sample. The heme and nonheme iron values obtained by the heme and nonheme iron methods were summed and compared to the total iron values obtained by the two total iron methods in Tables 5 and 6. The means represent the best estimates of the analyte in baked ground beef and the standard deviation represents intersample variability. Student's t test revealed no significant differences ($p>.05$) between the Hornsey (heme) + modified Schricker (nonheme) and the ferrozine (total) iron values in rare and medium-done beef, but significant difference ($p<.05$) was seen in well-done beef because of the overestimation of the heme iron by the Hornsey method. The AAS total iron values were significantly different ($p<.05$) from the total iron values obtained indirectly by the summation of the Hornsey (heme) and the modified Schricker (nonheme) iron values (Table 5). Similar

conclusions were made for the total iron values obtained by the summation of the Hornsey (heme) and the sodium pyrophosphate (nonheme) iron values (Table 6).

Table 6 - Comparison of Hornsey (heme) + sodium pyrophosphate (nonheme) iron determinations with ferrozine and AAS (total) iron determinations in baked ground beef patties (μg/g)

	Heme + Nonheme	Total Iron			
Sample[a]	Hornsey + $Na_4P_2O_7$[b]	Ferrozine[c]	diff[e]	AAS[d]	diff[e]
Rare	33.9 ±1.4	35.3 ±1.4	1.48(NS) ±2.21	30.7 ±1.6	-3.11(SD) ±2.22
Medium	35.3 ±1.3	34.7 ±2.0	-0.56(NS) ±1.97	29.7 ±2.2	-5.82(SD) ±2.07
Well-done	40.6 ±1.9	35.2 ±2.3	-5.68(SD) ±2.61	27.6 ±1.3	-13.33(SD) ±1.54

[a]Samples were baked to an internal temperature of 60°C (rare), 74°C (medium) or 80°C (well-done).

[b]Means and standard deviations obtained by the summation of values of the Hornsey and sodium pyrophosphate methods (n=5, each sample was analyzed in triplicate).

[c]Ferrozine means and standard deviations of 5 samples. Each sample was analyzed in triplicate.

[d]AAS means and standard deviations of 5 samples. Each sample was analyzed in triplicate.

[e]Mean difference (between heme+nonheme and total) and standard deviation of the mean difference. Student's t test at the 95% confidence level; NS=no significant difference, SD=significantly different.

The total iron in ground beef baked rare, medium and well-done determined by the summation of the heme iron and nonheme iron values, and the two total iron methods, ferrozine and AAS is presented in Fig. 1. The overestimation of the heme iron in well-done beef by the Hornsey method, and the underestimation of the total iron in rare, medium, and well-done beef by the AAS method can be seen clearly.

The individual coefficients of variation for each sample are given in the appendix.

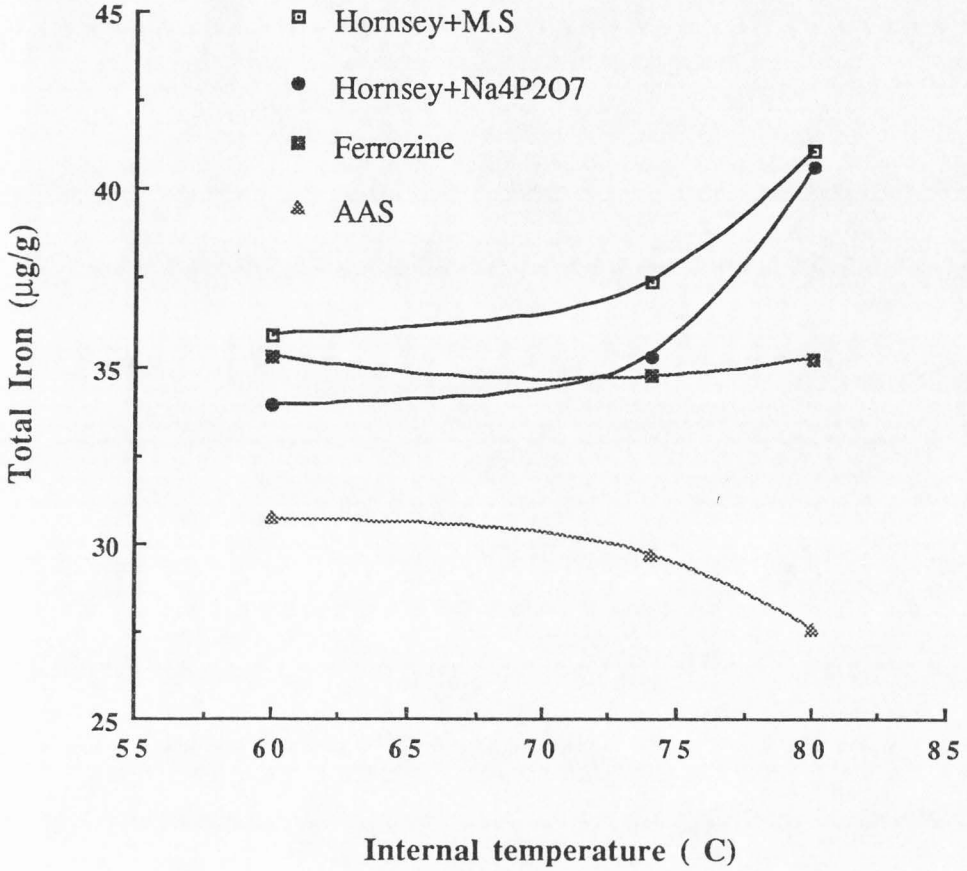

Figure 1 - Total iron content of ground beef baked rare, medium, and well-done determined by Hornsey+modified Schricker, Hornsey+sodium pyrophosphate, ferrozine, and AAS methods.

Non-interference of the Mineral Matrix

The total iron recoveries seen with the AAS method indicate that there are interferences with the ability of the AAS to detect all of the iron present in the sample. To investigate the effect of the sample mineral matrix on the accuracy of the AAS, a mineral matrix using $CaCl_2$, MgO, K_2CO_3, NaH_2PO_4, and $Zn(C_2H_3O_2)_2$ was made. The mineral matrix was similar to the mineral composition (except iron) of baked, ground beef (food composition tables, Rand et al., 1987). Known concentrations of iron were added to the matrix, and it was wet ashed and treated the same way as the beef samples. The prepared solution was analyzed for total iron by the AAS. The standard curve obtained in this way was similar to the standard curve obtained by using a standard iron stock solution (Fig. 2). This shows that the low accuracy of the AAS method may not be due to the sample mineral matrix.

Experiment Three

This experiment was conducted to determine the specificity and precision of the methods by testing the capability of the methods to detect varying concentrations of heme and nonheme iron. Beef liver is rich in both heme and nonheme iron. Catfish is a poor source of iron, but expressed as a percentage of total iron, catfish has a greater percentage of nonheme than beef liver. Thus, two sources - one of which has a high percentage heme and the other which has a higher percentage nonheme were chosen for the experiment.

The data on heme and nonheme iron analysis of mixtures of varying proportions of beef liver and catfish determined by the heme and nonheme methods are presented in Table 7. As the proportions of beef liver to catfish decreased, the heme iron content of the mixture decreased from a mean value of 46.2 μg/g (100% beef liver) to 3.0 μg/g (100%

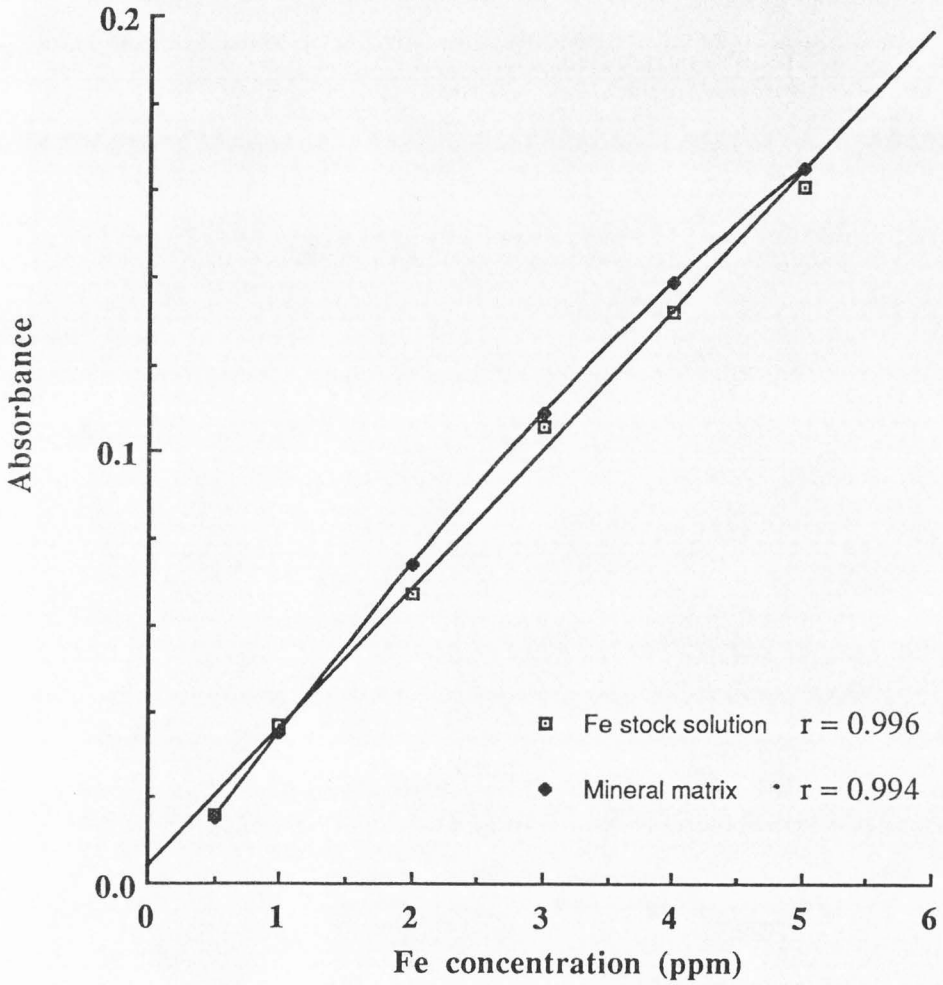

Figure 2 - Comparison of the AAS standard curves obtained with standard iron stock solution and with a mineral matrix background.

catfish). Nonheme iron decreased similarly. As determined by the modified Schricker method, the values were 45.2 µg/g (100% beef liver) and 7.2 µg/g (100% catfish). The sodium pyrophosphate extraction method gave nonheme iron values that were similar ($p>.05$) to those obtained by the modified Schricker method. The mean values obtained by the sodium pyrophosphate extraction method were 44.5 µg/g (100% beef liver) and 6.9 µg/g (100% catfish).

Table 7 - Total, heme and nonheme iron (µg/g fresh weight) in mixtures of varying proportions of beef liver (BL) and catfish (CF) determined by the ferrozine, Hornsey, modified Schricker and sodium pyrophosphate extraction methods

Sample		Total	Heme	Nonheme			
BL%/CF% F-test[d]	% moisture	Ferrozine[a]	Hornsey[b]	M. Schricker[b]	$Na_4P_2O_7$[b]	diff[c]	
100/0	69.6 ±0.8	89.5 ±0.9	46.2 ±3.7	45.2 ±4.8	44.5 ±5.9	0.7	NS
75/25		69.7 ±0.7	33.7 ±1.0	34.7 ±1.3	36.5 ±3.0	1.8	NS
50/50		49.9 ±0.5	22.2 ±0.7	22.8 ±3.1	24.5 ±2.0	1.7	NS
25/75		30.0 ±0.3	14.5 ±0.7	15.1 ±1.4	17.5 ±2.4	2.4	NS
0/100	73.1 ±1.4	10.2 ±0.3	3.0 ±0.3	7.2 ±0.3	6.9 ±0.6	0.3	NS

[a]Means and standard deviations of one liver and one catfish sample analyzed as 6 replicates. This data was used to compute the total iron content of the mixtures.
[b]Means and standard deviations of 3 samples; each sample was analyzed in triplicate.
[c]Difference between the mean nonheme iron values obtained by the modified Schricker and sodium pyrophosphate methods.
[d]F-test performed at the 95% confidence level; NS=no significant difference.

A two-way ANOVA revealed no significant differences ($p>.05$) between the mean nonheme iron values obtained by the two nonheme methods; however, the nonheme iron content was significantly different ($p<.05$) among mixtures. Between 47.3% to 56.5% of

iron in liver and 27.2% to 31.1% of iron in catfish are in the heme form. Also, the total iron content of beef liver and catfish obtained by the summation of the heme and nonheme iron values were similar to the total iron values determined by the ferrozine method (Table 7) and are consistent with the food composition tables (Rand et al., 1987).

The mean heme and nonheme iron values were plotted against the beef liver concentrations in the mixture. The correlation coefficients were 0.994 for the Hornsey method (Fig. 3), 0.991 for the modified Schricker method (Fig. 4) and 0.995 for the sodium pyrophosphate method (Fig. 5). These correlations suggest that the mixtures did not affect the extraction procedures used; they worked well at analyte concentrations ranging from 3.0 μg/g to 46.2 μg/g, on an average.

The individual coefficients of variation for each sample are given in the appendix.

NIST Reference Materials

Another way of establishing the accuracy, specificity, and precision of an analytical technique is to use certified reference materials analyzed by another laboratory. External standards in the form of Certified Reference Materials provide the most comprehensive means for establishing analytical competence and accuracy (Parr, 1985). The National Bureau of Standards provides reference materials intended primarily for the use of calibrating instrumentation and evaluating the reliability of analytical methods from the determination of major, minor and trace elements. The certified values for the element of interest are based on results obtained by reference methods of known accuracy and performed by two or more analysts or, alternately from results obtained by two or more independent, reliable analytical methods (Rasberry, 1985).

NIST standards were analyzed for total iron using the ferrozine and the AAS methods. The comparison of the data obtained by the ferrozine and AAS against the NIST-certified concentrations is presented in Table 8. Ferrozine values were consistently within

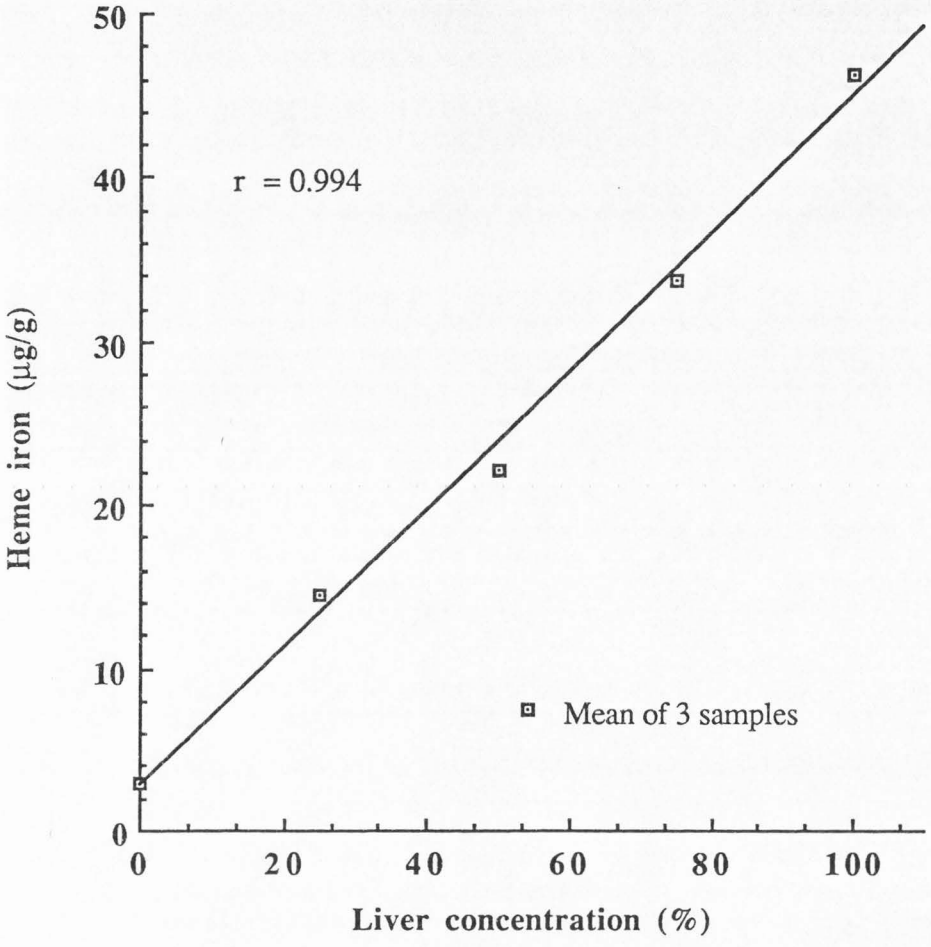

Figure 3 - Heme iron in beef liver/catfish mixture determined by the Hornsey method.

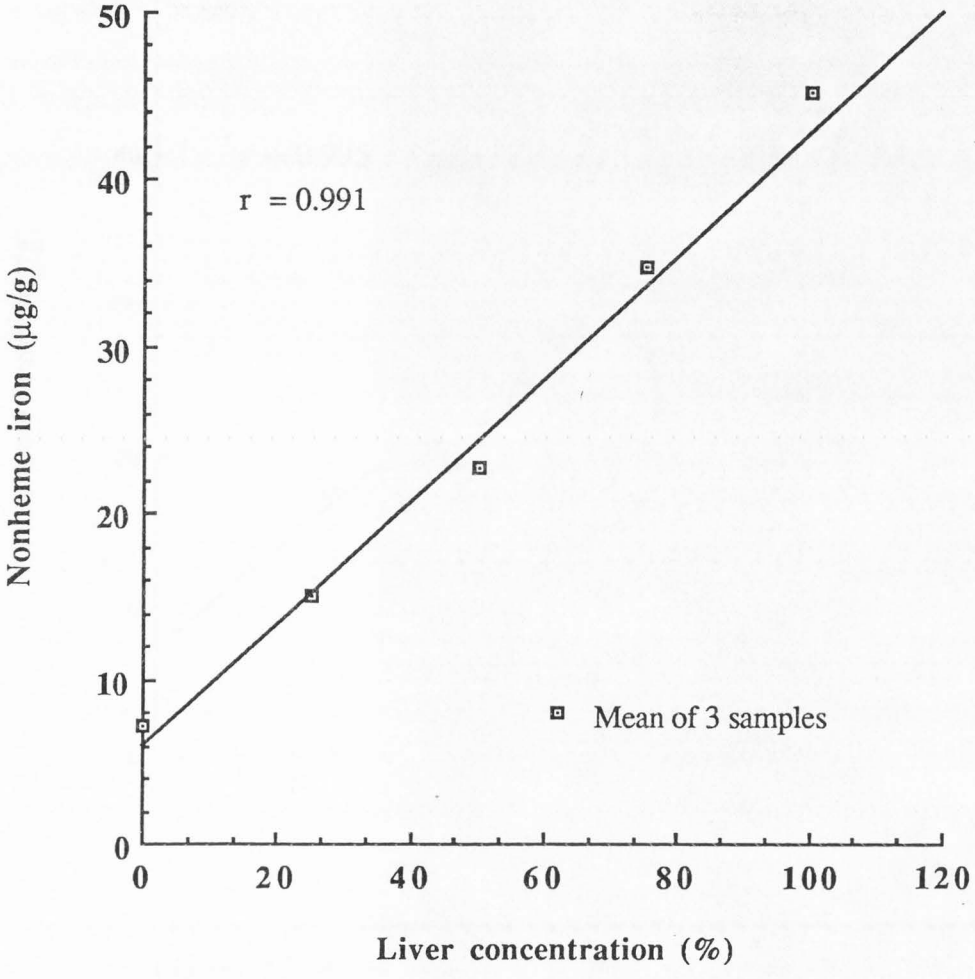

Figure 4 - Nonheme iron in beef liver/catfish mixture determined by the modified Schricker method.

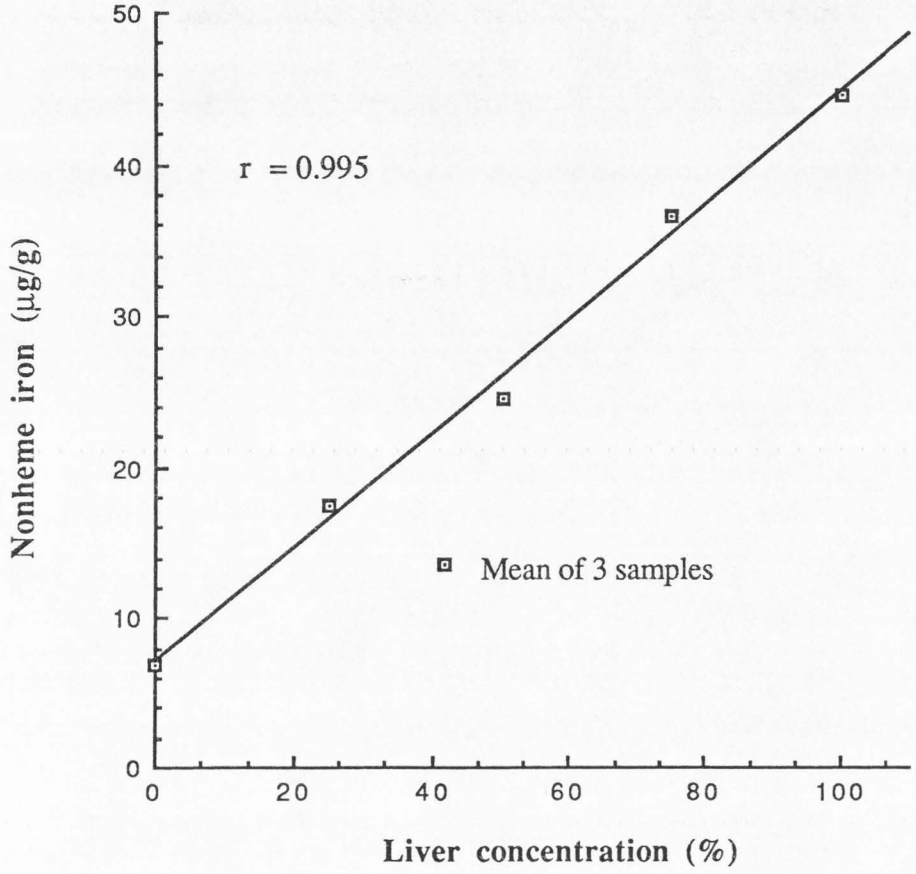

Figure 5 - Nonheme iron in beef liver/catfish mixture determined by the sodium pyrophosphate method.

the quoted NIST uncertainties, and the AAS values were outside the quoted NIST uncertainties. The non-fat dry milk iron content of 2.1 μg/g is a non-certified value.

Student's t test showed no significant difference ($p>.05$) between NIST-certified values and values obtained by the ferrozine method; however, significant differences ($p<.05$) exist between NIST-certified concentrations and AAS values. This further establishes the reliability and accuracy of the ferrozine technique and the lack of accuracy of the atomic absorption spectrophotometry.

During the course of my experimentation, I found that the ferrozine color reagent has some drawbacks which can prove to be frustrating for a beginner. The food sample used for total iron analysis using the ferrozine reagent is wet ashed and the ash is dissolved in a weak acid solution to keep the iron in a soluble form. Before the addition of the ferrozine reagent, the pH of the sample solution is adjusted to the working pH range of

Table 8 - Comparison of ferrozine and atomic absorption spectrophotometry (AAS) iron measurements (μg/g) with National Institute of Science and Technology (NIST) certified concentrations (μg/g)

Sample	No. obs.	NIST[a]	Ferrozine[b]	diff[d]	AAS[c]	diff[d]
SRM 1567 Wheat flour	5	18.3 ±1.0	17.8 ±0.9	-0.5	13.4 ±1.3	-4.9
SRM 1577 Bovine liver	9	268 ±8	264 ±6	-4	214 ±15	-54
NDM[f] (non-certified)	3	2.1	4.0 ±0.3	e	2.3 ±1.0	e
Student's t test[g]				NS		SD

[a]NIST-certified concentrations (mean ± standard deviation).
[b]Ferrozine means and standard deviations.
[c]AAS means and standard deviations.
[d]Difference between the mean value obtained by the method and NIST certified value.
[e]No statistical tests performed because it is a non-certified value.
[f]Non-fat dry milk.
[g]Student's t test performed at the 95% confidence level; NS=no significant difference and SD=significantly different.

ferrozine with diluted NH_4OH. Ferrozine is sensitive to pH and this could pose a problem as a source of experimental error.

The use of bathophenanthroline color reagent for the analysis of iron in food samples is common. It has a working pH range (2.0 to 9.5) broader than ferrozine (2.0 to 6.0) (Derman et al., 1989) and is less sensitive to pH changes than ferrozine. Thus, the use of the bathophenanthroline reagent could be simpler and more convenient. However, the ferrozine color reagent is cheaper than bathophenanthroline. Student's t test indicates no significant differences ($p>.05$) between values obtained by the batho reagent and NBS-certified concentrations (Table 9).

Table 9 - Comparison of total iron concentrations obtained using the bathophenanthroline color reagent with NIST-certified concentrations (μg/g)

Sample	No. obs.	NBS[a]	Batho[b]	diff[c]	t-test[d]
SRM 1567 Wheat flour	9	18.3 ±1.0	18.4 ±0.7	0.1	NS
SRM 1577 Bovine liver	3	268 ±8	265 ±8	-3	NS
NDM[f] (non-certified)	3	2.1	4.2 ±0.3	e	

[a]NIST-certified concentrations (mean ± standard deviation).
[b]Means and standard deviations obtained using the bathophenanthroline color reagent.
[c]Difference between mean batho values and NIST-certified concentrations.
[d]Student's t test performed at 95% confidence level; NS=no significant difference.
[e]No statistical tests performed because it is a non-certified value.
[f]Non-fat dry milk.

DISCUSSION

Heme Iron Method

The accuracy, specificity and precision of the Hornsey method were determined in three experiments through heme iron recoveries of hemoglobin spikes in raw beef and raw chicken samples, heme iron analysis of ground beef and beef liver/catfish mixtures.

Spike recoveries that were not different ($p>.01$) from 100% showed that the Hornsey method is an accurate technique with good specificity (Table 1). Also, there were no significant differences ($p>.01$) in spike recoveries between the 0.01 g and 0.02 g hemoglobin spikes in both raw beef and raw chicken samples. This suggests that the heme iron concentration did not affect the ability of the method to completely extract the heme iron present in the samples.

Igene et al. (1979) observed that cooking releases nonheme iron from heme pigments. Schricker and Miller (1983) suggest that the increase in nonheme iron concentration occurs due to the release of iron from the heme complex of myoglobin and hemoglobin and that oxidative cleavage of the porphyrin ring is involved. Chen et al. (1984) concluded that both final temperature and rate of heating influenced the release of nonheme iron from meat heme pigments. This fact was confirmed by Buchowski et al. (1988); nonheme iron increased with cooking temperature. The data presented in Table 4 are consistent with these findings. The heme iron content of ground beef decreased ($p<.05$) from rare to medium-done as shown by the values obtained by the Hornsey method.

Also, the heme iron content in rare- and medium-done ground beef determined by the Hornsey method was almost identical to the total iron content determined by the ferrozine method minus the nonheme iron content determined by the two nonheme methods (Tables 5 and 6). This indicates that the Hornsey method has good specificity and

accurately. However, the heme iron content in well-done ground beef was higher ($p<.05$) than the value obtained by subtracting the nonheme iron determined by the two nonheme methods from the total iron determined by the ferrozine method. This was due to the overestimation of the heme iron content in well-done ground beef that may be due to browning of the meat sample which could contribute to the color of the filtrate and thereby increase its absorbance value (Fig. 1). It is concluded that the Hornsey method is not reliable for analysis of heme iron in well-done ground beef. Future work with the Hornsey method should be aimed at finding out whether the overestimation of the heme iron content in well-done ground beef can be solely attributed to the browning of the meat samples.

The heme iron values of raw beef and chicken (Table 1), heme iron plus nonheme iron in beef baked rare and medium, beef liver and catfish (Table 7) were consistent with the published values (Schricker et al., 1982; Cook and Monsen, 1976). This was another means of validating the Hornsey method.

The accuracy and specificity of the Hornsey method was also established through the analysis of beef liver/catfish mixtures (Table 7). The heme plus nonheme iron values equalled the total iron values determined by the ferrozine method and are consistent with the food composition tables (Rand et al., 1987). As the liver concentration increased, the heme iron content of the mixture increased; a correlation coefficient of 0.994 (Fig. 3) was obtained. The Hornsey method was able to accurately extract as low as 30 μg (in 10 g catfish) and up to 323 μg heme iron (256 μg heme in 10 g raw beef + 67 μg heme in 0.02 g hemoglobin) in a given sample, the lowest and highest heme iron concentrations studied.

The precision of the Hornsey method is presented as coefficients of variation in the appendix. The overall coefficient of variation for the Hornsey method was 7.1%.

The Hornsey method is one of the most common methods used routinely in laboratories for quantitative determinations of heme iron in food samples (Jansuittivechakul et al., 1986; Buchowski et al., 1988 & 1989; Warriss et al., 1990; Kalpalathika et al., 1991a). This study has shown that the Hornsey method is a reliable, consistent and

accurate technique except when analyzing well-done beef samples. Since only one reagent is used as the extraction solvent, it is relatively safe, fairly simple and rapid. Also, the sources of contamination are minimized thus, allowing for accurate analysis of low concentrations of heme iron.

Nonheme Iron Methods

The accuracy, specificity and precision of nonheme iron methods were determined in this study by nonheme iron analysis of baked ground beef and beef liver/catfish mixtures.

The best way to test for complete extraction is to compare the method in question with an indirect one in which the nonheme is calculated from the difference between the total iron and heme iron (Bruckmann and Zondek, 1940). The nonheme iron values in rare- and medium-done ground beef determined by both modified Schricker and sodium pyrophosphate methods were almost identical to the nonheme iron values obtained indirectly by subtracting the heme iron content determined by the Hornsey method from the total iron content determined by the ferrozine method. This indicates that both the nonheme iron methods tested have good specificity and are accurate techniques. However, the values differed ($p<.05$) for well-done ground beef because of the overestimation of the heme iron content by the Hornsey method (Fig. 1).

The nonheme iron in ground beef increased ($p<.05$) with baking as shown by the values obtained by the two nonheme iron methods (Table 4). This confirms the findings of other workers (Igene et al., 1979; Schricker and Miller, 1983; Chen et al., 1984; Buchowski et al., 1988) that the nonheme iron content increases with increases in cooking temperatures.

The accuracy and specificity of the nonheme iron methods was also established by analyzing beef liver/catfish mixtures for nonheme iron. The total iron contents of beef liver and catfish obtained by the summation of the heme and nonheme iron values were close to

the total iron values determined by the ferrozine technique (Table 7) and are consistent with the food composition tables (Rand et al., 1987).

Nonheme iron content determined by the two methods showed an increase in nonheme iron content of the beef liver/catfish mixtures with a increase in beef liver concentration in the mixture. The correlation coefficients obtained by plotting nonheme iron content against the beef liver concentration in the mixture were 0.991 (Fig. 4) for the modified Schricker method and 0.995 (Fig. 5) for the sodium pyrophosphate method. This was one of the bases for validating the two nonheme iron methods.

The modified Schricker method was able to accurately analyze 36 μg (in 5 g catfish) to 226 μg (in 5 g beef liver) of nonheme iron in a given sample, the lowest and highest levels of nonheme iron studied using the modified Schricker method. The sodium pyrophosphate method worked well at nonheme iron concentrations of 6.9 μg (in 1 g catfish) to 44.5 μg (in 1 g beef liver), the lowest and highest nonheme iron concentrations studied using the sodium pyrophosphate method.

The two nonheme iron methods studied, modified Schricker method and sodium pyrophosphate method, are quite different from each other in their extraction procedures. However, the nonheme iron values obtained by these two methods were not different ($p>.05$) in all the samples studied. This suggests that the methods are appropriate and consistent in analyzing the nonheme iron in meats.

The precisions of the modified Schricker and the sodium pyrophosphate methods are presented as coefficients of variation (appendix). The overall coefficients of variation were 8.2% for the modified Schricker method and 7.6% for the sodium pyrophosphate method.

The modified Schricker and sodium pyrophosphate methods are routinely used for nonheme iron analysis in food samples; however, the Schricker method (Chen et al., 1984; Buchowski et al., 1988 & 1989) with the modifications suggested by Rhee and Ziprin (Bowers et al., 1989; King et al., 1990) is more common. This study showed that the two

nonheme methods tested -- modified Schricker and sodium pyrophosphate methods are reliable and accurate. Both these methods have the advantages of being simple, easy and require low operator time. The major drawback of both the methods is that they are not rapid techniques.

Of the two methods tested, the modified Schricker method would be the preferred method because it is less cumbersome. The sodium pyrophosphate method requires greater time for color development because of the slow release of ferric iron from ferric pyrophosphate formed during extraction. Moreover, the solubility of ferric pyrophosphate only at an acidic pH may cause problems because of the alkalinity of the sodium pyrophosphate reagent.

Total Iron Methods

The accuracy, specificity and precision of the total iron methods were determined in this study through total iron analysis of NIST standards and hemoglobin, total iron recoveries of hemoglobin spikes and total iron analysis of baked beef samples.

Total iron analysis of NIST standards (Table 8) by ferrozine and AAS methods showed that the ferrozine values were within the certified concentrations but the AAS values were lower ($p<.05$). The total iron values in hemoglobin as determined by the ferrozine and the AAS methods were 3.35 mg/g and 3.25 mg/g respectively (Table 2). Neither of these values were differed ($p>.05$) from the reference figure of 3.35 mg/g (Mahoney and Hendricks, 1982). This suggests that the ferrozine technique is accurate and specific to total iron while the AAS method seems inconsistent.

Total iron recoveries from hemoglobin spikes in raw beef and raw chicken samples were not different ($p>.01$) from 100% for the ferrozine method, but the AAS method gave values which were lower than 100% (Table 3). This further establishes the accuracy and specificity of the ferrozine technique and the lack of accuracy of the AAS method. This study also showed that the spike recoveries of 0.01 g and 0.02 g hemoglobin in both beef

and chicken were not different ($p>.05$) indicating that the total iron concentrations of the sample did not affect the accuracies of the methods.

The total iron content in raw beef and raw chicken determined by the ferrozine method were consistent with the food composition tables (Rand et al., 1987) while the AAS values were not (Table 2). In baked beef, there were no differences ($p>.05$) in total iron contents of rare, medium and well-done beef, but the AAS values were lower than the ferrozine values (Table 4) which were in agreement with the food composition tables (Rand et al., 1987). The total iron in beef liver and catfish determined by the ferrozine method are consistent with the food composition tables (Rand et al., 1987). This provides the basis for validating the ferrozine technique while stating that the AAS method was not able to detect all of the iron present in the sample.

The accuracy and specificity of the ferrozine technique can also be established by the data in Tables 5 and 6. The total iron values in rare- and medium-done beef as determined by the ferrozine method were almost identical to the heme iron value determined by the Hornsey method plus the nonheme iron value determined by the two nonheme iron methods. The AAS values were significantly lower ($p<.05$) than the heme plus nonheme values. In well-done beef, the heme plus nonheme values were significantly higher ($p<.05$) than the ferrozine or AAS total iron values. This was because of the overestimation of the heme iron content of well-done beef by the Hornsey method (Fig. 1).

The precisions of the two total iron methods are presented as coefficients of variation (appendix). The overall coefficients of variation among triplicate analyses of the same sample were 5.0% for the ferrozine method and 5.8% for the AAS method. This confirms the findings of Davies et al. (1972) that the AAS determinations have more variability than the colorimetric determinations. Also the C.V. for the AAS method obtained in this study is close to the earlier reported value of 6.5% (De Ruig, 1986).

The AAS method gave total iron values that were consistently lower than the ferrozine total iron values in all samples tested in this study except in hemoglobin. This

may be due to the homogeneity of the hemoglobin sample; the presence of other minerals in food samples interferes with the ability of the atomic absorption spectrophotometer to detect a given concentration of iron (Favier et al., 1985; AA Methods Manual, 1975). The same accuracy was not seen when a heterogeneous product such as beef or chicken was used. However, the interference may not be due to the mineral matrix of the meat sample (Fig. 2). Further research in this area should consider factors such as interference due to the non-mineral composition of meats and the effect of acetylene to oxygen ratio on the detection ability of the AAS.

The ferrozine method (Buchowski et al., 1988 & 1989; Zhang and Mahoney, 1990 & 1991; Kalpalathika et al., 1991b) and the AAS method (Schricker et al., 1982; Jansuittivechakul et al., 1986; Nielson et al., 1988; Bowers et al., 1989; Allred et al., 1990; King et al., 1990; Marti et al., 1990; Falandysz, 1991) are the most common methods used today for routine analysis of total iron in food samples. This study showed that the ferrozine method is consistent, reliable and accurate. It is easy to use, rapid, safe and inexpensive. On the other hand, the AAS method has been seen to lack accuracy; it was able to detect, on an average, only 80 to 85% of the total iron present in the sample.

CONCLUSION

The validity of some heme, nonheme and total iron methods was determined using spikes of hemoglobin, ground beef baked to different degrees of doneness, proportional beef liver:catfish mixtures and NIST reference materials.

The Hornsey method for quantifying heme iron in meats was seen to produce accurate results; however, it overestimated the heme iron content of well-done ground beef possibly due to the browning of meat baked at that temperature.

The two nonheme iron methods tested -- modified Schricker and sodium pyrophosphate methods -- were validated as reliable techniques via nonheme iron determinations in meat, liver and fish samples. Both methods have the attributes of being simple, sensitive, require low operator time, and are able to achieve complete extraction of nonheme iron. Both of these methods have the disadvantage of not being rapid techniques.

Through the determinations of iron in hemoglobin spiked ground beef and unspiked ground beef samples and the use of NIST reference materials, it was demonstrated that of the two total iron methods tested, the ferrozine technique is more reliable and accurate than the AAS method. The atomic absorption spectrophotometer consistently underestimated the total iron values and was able to detect, on an average, only 80-85% of the total iron present in the sample. The mineral matrix of the meat sample did not interfere with the iron detection ability of the AAS instrument.

Future research in this area should be aimed at investigating the effect of browning on the accuracy of the Hornsey method and the problems in the determination of total iron by the atomic absorption spectrophotometer.

REFERENCES

Adler, A.D. and George, P. 1965. Determination of iron in heme compounds. I. Hemin. Anal. Biochem. 11:159-163.

Ahn, D.U. and Maurer, A.J. 1990. Poultry meat color: Kinds of heme pigments and concentrations of the ligands. Poultry Science. 69:157-165.

Allred, L.C., Hendricks, D.G., Mahoney, A.W., Zhang, D., and Bell, D.E. 1990. Protein quality and iron bioavailability of mechanically and hand-deboned turkey meat fed to rats. Poultry Science. 69:341-347.

Alvarez, R. 1984. NBS standard reference materials for food analysis. In "*Modern Methods of Food Analysis*," K.K. Stewart (Ed.), p.87. AVI Publishing Co., Westport, CN.

AOAC (Official Methods of Analysis of the Association of Official Analytical Chemists) 1990. S.Williams (Ed.) 20th edition. AOAC Inc., Arlington, VA.

Arthur, C.K. and Isbister, J.P. 1987. Iron deficiency. Misunderstood, misdiagnosed and mistreated. Drugs. 33:171-182.

Atomic absorption methods manual, 1975. Vol.1. Standard conditions for flame operation. Instrumentation Laboratory Inc., Analytical instrument division, Wilmington, MA.

Bezkorovainy, A. 1989. Biochemistry of nonheme iron in man. II. Absorption of iron. Invited Review Article. Clin. Physiol. Biochem. 7:53-69.

Bothwell, T.H., Charlton, R.W., Cook, J.D., and Finch, C.A. 1979. "Iron Metabolism in Man," p. 256. Blackwell Scientific Publications, Oxford, England.

Bowers, J.A., Craig, J., and Williams, J.C. 1989. Sensory characteristics, texture, color, and selected nutrient content of veal muscle. J. Food Sci. 54(6):1444-1470.

Bruckmann, G. and Zondek, S.G. 1940. An improved method for the determination of non-hemin iron. J. Biol. Chem. 135:23-30.

Buchowski, M.S., Mahoney, A.W., Carpenter, C.E., and Cornforth, D.P. 1988. Heating and the distribution of total and heme iron between meat and broth. J. Food Sci. 53(1):43-45.

Buchowski, M.S., Mahoney, A.W., and Kalpalathika, M.P.V. 1989. Nonheme iron absorption, apparent iron absorption and hemoglobin regeneration efficiency in anemic and normal rats fed with dietary heme and nonheme iron at various levels. Nutr. Res. 9:773-783.

Cameron, B.F. 1965. Determination of iron in heme compounds. II. Hemoglobin and myoglobin. Anal. Biochem. 11:164-169.

Caraway, W.T. 1963. Macro and micro methods for the determination of serum iron and iron-binding capacity. Clin. Chem. 9(2):188-199.

Carter, P. 1971. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal. Chem. 40:450-458.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H., and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implications in oxidation. J. Food Sci. 49:581-584.

Clegg, M.S., Keen, C.L., Lonnerdal, B., and Hurley, L.S. 1981a. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet ashing. Biol. Trace Element Res. 3:107-115.

Clegg, M.S., Keen, C.L., Lonnerdal, B., and Hurley, L.S. 1981b. Influence of ashing techniques on the analysis of trace elements in biological samples. II. Dry ashing. Biol. Trace Element Res. 3:237-244.

Cook, J.D. 1983. Determinants of nonheme iron absorption in man. Food Technol. October, 124-126.

Cook, J.D. and Monsen, E.R. 1976. Food iron absorption in human subjects. III. Comparison of the effects of animal proteins on nonheme iron absorption. Am. J. Clin. Nutr. 29:859-867.

Crosby, W.H., Munn, J.I., and Furth, F.W. 1954. Standardizing a method for clinical hemoglobinometry. U.S. Armed Forces Medical Journal. V(5): 693-703.

Davies, I.M., Bush, K., and Motzok, I. 1972. Determination of iron in low-iron diets by colorimetric and atomic absorption procedures. J. AOAC. 55(6):1206-1210.

De Ruig, W. 1986. Atomic absorption spectrometric determination of calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc in animal feeding stuffs: interlaboratory collaborative studies. J. AOAC. 69(6):1009-1013.

DeMaeyer, E.D. 1985. Fortification of Foods. In "*Food Science and Technology - A series of monographs,*" F.M. Clydesdale and K.L. Weimer (Ed.). Academic Press, Orlando, FL.

DeMaeyer, E.D. and Adiels-Tegman, M. 1985. World Health Statist. Q., 38:302.

Derman, D.P., Green, A., Bothwell, T.H., Graham, B., McNamara, L., MacPhail, A.P., and Baynes, R.D. 1989. A systematic evaluation of bathophenanthroline, ferrozine and ferene in an ICSH-based method of the measurement of serum iron. Ann. Clin. Biochem. 26:144-147.

Duffy, J.R. and Gaudin, J. 1977. Copper interference in the determination of iron in serum using ferrozine. Clin. Biochem. 10(3):122-123.

Fairbanks, V.F., Fahey, J.L., and Beutler, E. 1971. "Clinical Disorders of Iron Metabolism." 2nd ed. Grune & Stratton, Inc., New York, NY.

Falandysz, J. 1991. Manganese, copper, zinc, iron, cadmium, mercury and lead in muscle meat, liver and kidneys of poultry, rabbit and sheep slaughtered in the northern part of Poland, 1987. Food Additives and Contaminants. 8(1):71-83.

Favier, A., Ruffieux, D., and Arnaud, J. 1985. Matrix effects on serum trace elements analysis by flameless atomic absorption. Nutr. Research. Suppl. I: 63-70.

Foy, L.A., Williams, H.L., Cortell, S., and Conrad, M.E. 1967. A modified procedure for the determination of nonheme iron in tissue. Anal. Biochem. 18:559-563.

Friel, J.K. and Ngyuen, C.D. 1986. Dry- and wet-ashing techniques compared in analyses for zinc, copper, manganese, and iron in hair. Clin. Chem. 32(5):739-742.

Gibbs, C.R. 1976. Characterization and application of ferrozine iron reagent as a ferrous iron indicator. Anal. Chem. 48(8):1197-1201.

Hallberg, L. 1981. Bioavailability of dietary iron in men. Ann. Rev. Nutr. 1:123-127.

Hallberg, L. and Bjorn-Rasmussen, E. 1972. Determination of iron absorption from whole diet: a new pool model using two radioiron isotopes given as heme and nonheme iron. Scand. J. Haematol. 9:193-197.

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

Harnley, J.M. and Wolf, W.R. 1984. Atomic spectroscopy for inorganic elements in foods. In "*Analysis of Foods and Beverages, Modern Techniques*," G. Charalambous (Ed.) p.506-551. Academic Press Inc., New York, NY.

Harnley, J.M. and Wolf, W.R. 1985. Simultaneous multielement atomic absorption spectrometry for analysis of biological materials. Nutr. Research. Suppl. I: 77-79.

Hill, A.D., Patterson, K.Y., Veillon, C., and Morris, E.R. 1986. Digestion of biological materials for mineral analyses using a combination of wet and dry ashing. Anal. Chem. 58(11):2340-2342.

Hornsey, H.C. 1956. The color of cooked cured pork. I. Estimation of the nitric oxide-heme pigments. J. Sci. Food Agric. 7:534-540.

Igene, J.O., King, J.A., Pearson, A.M., and Gray, J.I. 1979. Influence of heme pigments, nitrite, and nonheme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27(4):838-842.

Jansuittivechakul, O., Mahoney, A.W., Cornforth, D.P., Hendricks, D.G., and Sisson, D.V. 1986. Effect of heat treatment on meat enhancement of dietary iron bioavailability of meat/ferrous sulfate and meat/hemoglobin mixtures fed to anemic rats. J. Food Sci. 51(2):263-267.

Kalpalathika, M.P.V., Clark, E.M., and Mahoney, A.W. 1991a. Heme iron content in selected ready-to-serve beef products. J.Agric.Food Chem. 39(6):1091-1093.

Kalpalathika, M.P.V., Mahoney, A.W., Whittaker, P., and Hendricks, D.G. 1991b. Incorporation of absorbed iron from different dietary sources into hemoglobin. Nutr. Res. 11:185-195.

Kawasaki, N., Ishigami, A., Tanimoto, T., and Tanaka, A. 1990. Determination of iron(III) ion using ion chromatography with electrochemical detection and its application to the assay of the ferroxidase activity of ceruloplasmin. J. Chromatography. 503:237-243.

Kerr, L.M.H. 1957. A method for the determination of non-heme iron bone marrow. Biochem. J. 67:627-630.

King, J., De Pablo, S., and Montes De Oca, F. 1990. Nonheme iron evaluation of a hemoglobin iron concentrate. J. Food Sci. 55(2):593-594.

Klein, B. 1971. Automated serum iron determinations. Clin. Toxicol. 4(4):631-639.

Labbe, R.F. and Nishida, G. 1957. A new method of hemin isolation. Biochem. Biophys. Acta. 26:437.

Ledward, D.A. 1974. On the nature of hematin-protein bonding in cooked meat. J. Fd. Technol. 9:59-68.

Lee, K. and Clydesdale, F.M. 1979. Quantitative determination of the elemental, ferrous, ferric, soluble, and complexed iron in foods. J. Fd. Sci. 44(2):549-554.

Mahoney, A.W. and Hendricks, D.G. 1982. Efficiency of hemoglobin regeneration as a method of assessing iron bioavailability in food products. In "*Nutritional Bioavailability of Iron*," C. Kies (Ed.) p.1-11. American Chemical Society: Washington, DC.

Marti, M.T., Saiz, M.P., Mitjavila, M.T., and Planas, J. 1990. Organ iron content in New Hampshire chickens. Biological Trace Element Research. 25:1-9.

Monsen, E.R., Hallberg, L., Layrisse, M., Hegsted, D.M., Cook, J. D., Mertz, W., and Finch, C.A. 1978. Estimation of available dietary iron. Am. J. Clin. Nutr. 31(1):134-141.

Morris, R.L. 1952. Determination of iron in water in the presence of heavy metals. Anal. Chem. 24(8):1376-1379.

Narayanan, S. and Lin, F.C. 1985. Sampling considerations in trace metal analysis. Nutr. Research. Suppl I: 59-62.

Nielson, K.K., Mahoney, A.W., and Rogers, V.C. 1988. X-ray fluorescence and atomic absorption spectrophotometry measurements of manganese, iron, copper, and zinc in selected foods. J. Agric. Food Chem. 36(6):1211-1216.

Olszon, E., Iaksson, B., Norrby, A., and Solvell, L. 1978. Food iron absorption in iron deficiency. Am. J. Clin. Nutr. 31:106-111.

Parr, R.M. 1985. Quality assurance of trace element analyses. Nutr. Research. Suppl I: 5-11.

Rand, W.M., Windham, C.T., Wyse, and B.W., Young, V.R. 1987. "Food Composition Data: A User's Prospective." The United Nations Library, New York, NY.

Rasberry, S.D. 1985. "National Bureau of Standards: Office of Standard Reference Materials." U.S. Dept. of Commerce, Washington, D.C.

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. J. Fd. Sci. 52(5):1174-1176.

Salinas, F., Sanchez, J.C.J., and Diaz, T.G. 1986. Spectrophotometric determination of iron in wines, foods, and minerals with 5,5-dimethyl-1,2,3,-cyclohexanetrione 1,2-dioxime 3-thiosemicarbazone. Anal. Chem. 58(4):824-827.

Schricker, B.R. and Miller, D.D. 1983. Effects of cooking and chemical treatment on heme and nonheme iron in meat. J. Food Sci. 48:1340-1345.

Schricker, B.R., Miller, D.D., and Stouffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. J. Food Sci. 47:740-743.

Sharma, D.C., Singh, P.P., and Khalsa, J.K. 1969. Determination of serum iron with dimethylglyoxime. Clin. Biochem. 2:439-445.

Skikne, B.S. 1988. Current concepts in iron deficiency anemia. Food Reviews International. 4(2):137-173.

Stewart, K.K. 1989. Data validation. J. Fd. Composition and Analysis. 2:91-92.

Stookey, L.L. 1970. Ferrozine - a new spectrophotometric reagent for iron. Anal. Chem. 42:771-774.

Stoskman, J.A. 1987. Iron deficiency anemia: have we come far enough? JAMA 258:1645-1647.

Thompsen, J.C. and Mottola, H.A. 1984. Kinetics of the complexation of iron(II) with ferrozine. Anal. Chem. 56(4):755-757.

Tompsett, S.L. 1934. Studies of the complexes of iron with various biological materials. Biochem. J. 28:1802-1806.

Tompsett, S.L. 1935. The copper and "inorganic" iron contents of human tissues. Biochem. J. 29:480-486.

Torrance, J.D. and Bothwell, T.H. 1968. A simple technique for measuring storage iron concentrations in formalinised liver samples. S. Afr. J. Med. Sci. 33:9-11.

Turnbull, A.L., Cleton, F., and Finch, C.A. 1962. Iron absorption. IV. The absorption of hemoglobin iron. J. Clin. Invest. 41:1897-1907.

Vuori, I.M., Varonen, E.R., and Hartiala, K. 1963. An improved method for iron determination in biological material. Scandinav. J. Clin. Lab. Investigation. 15:323-325.

Warriss, P.D. 1979. The extraction of heme pigments from fresh meat. J. Fd. Technol. 14:75-80.

Warriss, P.D., Brown, S.N., and Adams, S.J.M. 1990. Variation in heme pigment concentration and color in meat from British pigs. Meat Sci. 28:321-329.

Wickramasinghe, R.H. 1974. The investigation of acid-labile protein-iron using bathophenanthroline sulphonate. Clin. Biochem. 7:88-90.

Wolf, W.R. 1982. Trace element analysis in food. In "*Clinical, biochemical, and nutritional aspects of trace elements*," p. 427-446. Alan R. Liss, Inc., New York, NY.

Yang, J.Y., Yang, M.H., and Lin, S.M. 1990. Effect of wet decomposition methods on the determination of cobalt, copper, selenium, and zinc in biological samples using electrophoresis. Anal. Chem. 62(2):146-150.

Zhang, D. and Mahoney, A.W. 1990. Effect of iron fortification on quality of cheddar cheese. 2. Effects of aging and fluorescent light on pilot scale cheeses. J. Dairy Sci. 73(9):2252-2258.

Zhang, D. and Mahoney, A.W. 1991. Iron fortification of process cheddar cheese. J. Dairy Sci. 74(2):353-358.

APPENDIX

Table 10. Heme iron analysis of spiked and unspiked raw beef by the Hornsey method.

	Raw beef (μg/g fresh weight)	Spike of 0.01 g Hb (% recovery)	Spike of 0.02 g Hb (% recovery)
Sample 1	27.5	89.2	105.2
	27.2	107.8	127.0
	26.4	119.3	106.6
Average±SD	27.0±0.6	105.4±15.2	112.9±12.2
Sample 2	22.7	96.6	106.6
	24.3	131.1	105.2
	25.7	98.3	
Average±SD	24.2±1.5	108.7±19.4	105.9±1.0
Sample 3	25.2	84.4	99.1
	25.0	93.5	102.3
	25.8	86.9	
Average±SD	25.3±0.4	88.3±4.7	100.7±2.3
Sample 4	25.4	84.0	91.6
	24.7	88.1	93.7
	26.1	86.8	95.0
Average±SD	25.4±0.7	86.3±2.1	93.4±1.6
Sample 5	25.2	85.8	95.4
	25.7	87.3	92.2
	26.6	94.6	93.9
Average±SD	25.8±0.7	89.2±4.7	93.8±1.6
Overall-mean	25.6	95.6	101.1
C.V. (%)	4.65	14.41	9.54

Table 11. Total iron analysis of spiked and unspiked raw beef by the Ferrozine method.

	Raw beef (μg/g fresh weight)	Hemoglobin (mg Fe/g)	Spikes of 0.01 g Hb (% recovery)	Spikes of 0.02 g Hb (% recovery)
Sample 1	38.0	3.49	90.5	96.0
	36.2	3.36	98.5	103.0
	34.0	3.30	100.5	99.5
	35.9		98.9	
Average±SD	36.0±1.6	3.38±0.10	96.5±5.3	99.3±2.9
Sample 2	38.5	3.33	98.0	97.4
	34.9	3.36	100.0	96.2
	36.2	3.40	98.5	97.3
Average±SD	36.5±1.8	3.36±0.04	98.8±1.0	97.0±0.7
Sample 3	36.5	3.25	99.1	97.7
	36.6	3.32	102.6	99.6
	37.4	3.38	99.2	103.7
Average±SD	36.8±0.5	3.32±0.07	100.3±2.0	100.3±3.1
Sample 4	37.2	3.23	101.4	97.1
	36.0	3.36	98.4	99.7
	37.1	3.48	99.9	95.6
			96.0	
Average±SD	36.8±0.7	3.36±0.13	98.9±2.3	97.5±2.1
Sample 5	35.9	3.25	96.3	92.5
	36.9	3.22	97.7	101.1
	38.8	3.46	94.6	96.5
Average±SD	37.2±1.5	3.31±0.13	96.2±1.6	96.7±4.3
Overall mean	36.7	3.35	98.2	98.2
C.V. (%)	3.38	2.69	2.93	2.96

Table 12. Total iron analysis of spiked and unspiked raw beef by the AAS method.

	Raw beef (µg/g fresh weight)	Hemoglobin (mg Fe/g)	Spikes of 0.01 g Hb (% recovery)	Spikes of 0.02 g Hb (% recovery)
Sample 1	32.4	3.38	86.3	83.2
	34.5	3.22	95.0	95.3
	34.2	3.33	86.0	95.3
Average±SD	33.7±1.1	3.31±0.08	89.1±5.1	91.3±7.0
Sample 2	29.8	3.30	86.0	93.1
	32.3	3.28	86.2	90.9
	32.8	3.32	83.2	85.1
Average±SD	31.6±1.6	3.30±0.02	85.1±1.7	89.7±4.1
Sample 3	32.2	3.37	83.2	78.8
	26.6	3.32	83.2	77.0
	34.8	3.41	80.5	83.5
Average±SD	31.2±4.2	3.37±0.5	82.4±1.6	79.8±3.4
Sample 4	31.4	2.72	80.6	80.8
	31.0	2.73	85.3	82.4
	32.9	3.16	87.4	79.6
Average±SD	31.8±1.0	2.87±0.25	84.4±3.5	80.9±1.4
Sample 5	34.3	3.59	82.3	86.2
	28.4	3.32	82.9	86.8
	31.6	3.25	86.2	
Average±SD	31.4±3.0	3.39±0.18	83.8±2.1	86.5±0.4
Overall mean	31.9	3.25	85.0	85.6
C.V. (%)	7.24	7.08	4.13	7.06

Table 13. Heme iron analysis of spiked and unspiked raw chicken by the Hornsey method.

	Raw chicken (μg/g fresh weight)	Spike of 0.01 g Hb (% recovery)	Spike of 0.02 g Hb (% recovery)
Sample 1	6.4	92.3	95.9
	6.1	93.7	89.0
	6.0		79.7
Average±SD	6.2±0.2	93.0±1.0	88.2±8.1
Sample 2	5.0	101.3	85.5
	4.6	90.7	85.7
	5.5	92.6	92.8
Average±SD	5.0±0.5	94.9±5.7	88.0±4.2
Sample 3	5.9	92.8	92.5
	5.6	99.5	
	5.9	99.8	94.2
Average±SD	5.8±0.2	97.4±4.0	93.4±1.2
Sample 4	5.6	90.8	95.5
	5.7	89.4	103.9
	6.2	105.5	106.2
Average±SD	5.8±0.3	95.2±8.9	101.9±5.6
Sample 5	5.5	98.8	98.4
	4.7	94.3	104.6
	5.7	103.8	93.9
	5.7		
	5.8		
Average±SD	5.5±0.4	99.0±4.8	99.0±5.4
Overall mean	5.7	96.1	94.1
C.V. (%)	7.89	5.44	10.19

Table 14. Total iron analysis of spiked and unspiked raw chicken by the Ferrozine method.

	Raw chicken (μg/g fresh weight)	Hemoglobin (mg Fe/g)	Spikes of 0.01g Hb (% recovery)	Spikes of 0.02g Hb (% recovery)
Sample 1	11.5	3.49	103.9	105.7
	11.3	3.36	96.0	104.3
	12.5	3.30	87.4	99.6
Average±SD	11.8±1.6	3.38±0.10	95.8±8.3	103.2±3.2
Sample 2	12.8	3.33	95.4	98.6
	13.1	3.36	88.9	98.2
	12.4	3.40	107.1	98.8
Average±SD	12.8±0.4	3.36±0.04	97.1±9.2	98.5±0.3
Sample 3	11.0	3.25	92.1	97.8
	11.2	3.32	101.6	103.5
	10.7	3.38	97.5	97.6
Average±SD	11.0±0.3	3.32±0.07	97.1±4.8	99.6±3.4
Sample 4	14.4	3.23	96.6	96.2
	10.8	3.36	85.1	102.9
	12.9	3.48	81.3	95.8
Average±SD	12.7±1.8	3.36±0.13	87.7±8.0	98.3±4.0
Sample 5	10.0	3.25	97.2	96.2
	10.9	3.22	96.9	97.3
	9.0	3.46	108.6	97.4
Average±SD	10.0±1.0	3.31±0.13	100.9±6.7	97.0±0.7
Overall mean	11.6	3.35	95.7	99.3
C.V. (%)	10.43	2.69	8.16	3.21

Table 15. Total iron analysis of spiked and unspiked raw chicken by the AAS method.

	Raw chicken (µg/g fresh weight)	Hemoglobin (mg Fe/g)	Spikes of 0.01 g Hb (% recovery)	Spikes of 0.02 g Hb (% recovery)
Sample 1	8.6	3.38	91.8	82.3
	9.2	3.22	86.3	78.3
	8.9	3.33	88.5	80.9
			80.5	
Average±SD	8.9±0.3	3.31±0.08	86.8±4.8	80.5±2.0
Sample 2	8.1	3.30	81.8	83.1
	8.6	3.28	85.1	97.2
	9.3	3.32	88.3	85.5
Average±SD	8.7±0.6	3.30±0.02	85.1±3.3	88.6±7.5
Sample 3	9.3	3.37	83.5	83.3
	8.8	3.32	93.2	86.9
	8.0	3.41	84.9	88.0
Average±SD	8.7±0.7	3.37±0.5	87.2±5.2	86.1±2.5
Sample 4	8.9	2.72	91.9	84.2
	9.1	2.73	87.4	88.7
	8.1	3.16	84.1	87.1
Average±SD	8.7±0.5	2.87±0.25	87.8±3.9	86.7±2.3
Sample 5	8.9	3.59	88.2	84.4
	8.2	3.32	81.3	87.1
	8.6	3.25	89.1	89.0
Average±SD	8.6±0.4	3.39±0.18	86.2±4.3	86.8±2.3
Overall mean	8.7	3.25	86.6	85.7
C.V. (%)	5.05	7.08	4.46	5.11

Table 16. Heme, nonheme, and total iron (μg/g wet weight) analysis of rare-done ground beef.

	Heme iron		Nonheme iron	Total iron	
	Hornsey	M.Schricker	$Na_4P_2O_7$	Ferrozine	AAS
Sample 1	21.5	13.6	11.8	35.8	33.9
	20.2	13.4	12.1	36.2	29.9
	19.2	12.8	11.5	36.9	30.7
Average±SD	20.3±1.2	13.3±0.4	11.8±0.3	36.3±0.6	31.5±2.1
Sample 2	22.9	14.2	11.0	34.6	29.6
	22.2	10.9	11.1	35.1	33.6
		14.6	11.0	35.1	30.0
Average±SD	22.6±0.5	13.2±2.0	11.1±0.1	34.9±0.3	31.1±2.2
Sample 3	23.1	12.8	10.6	37.4	29.7
	23.2	13.2	10.2	35.1	28.2
	22.0	14.4	11.3	35.6	30.0
Average±SD	22.8±0.7	13.5±0.8	10.7±0.6	36.0±1.2	29.3±1.0
Sample 4	21.2	13.1	11.9	31.3	31.3
	22.3	14.0	12.4	35.6	31.6
	25.1	11.5	10.8	35.8	31.7
Average±SD	22.9±2.0	12.9±1.3	11.7±0.8	34.2±2.5	31.5±0.2
Sample 5	25.2	13.1	11.0	36.1	29.4
	24.2	14.2	10.2	35.1	29.1
	25.2	12.3	10.7	34.1	31.7
Average±SD	24.9±0.6	13.2±1.0	10.6±0.4	35.1±1.0	30.1±1.4
Overall mean	22.7	13.5	10.9	35.3	30.7
C.V. (%)	8.11	7.78	6.15	3.94	5.25

Table 17. Heme, nonheme, and total iron (μg/g wet weight) analysis of medium-done ground beef.

	Heme iron		Nonheme iron	Total iron	
	Hornsey	M.Schricker	$Na_4P_2O_7$	Ferrozine	AAS
Sample 1	21.4	15.3	13.1	36.2	30.2
	21.7	15.8	14.0	35.9	30.7
	21.3	15.0	13.6	35.9	30.8
Average±SD	21.5±0.2	15.4±0.4	13.5±0.5	36.0±0.2	30.6±0.3
Sample 2	25.5	16.1	14.3	35.9	32.6
	21.2	16.3	13.5	35.7	30.2
	20.9	16.5	14.5	34.8	29.7
Average±SD	22.5±2.6	16.3±0.2	14.1±0.5	35.5±0.6	30.8±1.6
Sample 3	21.1	16.0	14.0	35.7	29.7
	21.1	16.0	14.1	34.7	30.3
	21.2	14.8	13.5	30.9	29.9
	20.7				
Average±SD	21.0±0.2	15.6±0.7	13.8±0.3	33.8±2.5	30.0±0.3
Sample 4	21.0	16.4	14.6	36.2	29.1
	20.2		14.1	35.9	32.5
	20.1	16.2	15.2	36.4	31.7
Average±SD	20.4±0.5	16.3±0.1	14.6±0.5	36.2±0.3	31.1±1.8
Sample 5	21.5	15.7	14.0	33.9	25.7
	21.3		13.3	30.7	27.6
	21.5	16.1	13.6	31.8	25.0
Average±SD	21.4±0.1	15.9±0.3	13.6±0.3	32.1±1.6	26.1±1.3
Overall mean	21.4	15.9	13.9	34.7	29.7
C.V. (%)	5.70	3.27	4.03	5.71	7.31

Table 18. Heme, nonheme, and total iron (μg/g wet weight) analysis of well-done ground beef.

	Heme iron		Nonheme iron	Total iron	
	Hornsey	M.Schricker	$Na_4P_2O_7$	Ferrozine	AAS
Sample 1	24.6	17.1	16.9	37.9	29.7
	24.5	16.8	16.9	37.5	26.9
	18.7	17.4	17.3		
Average±SD	22.6±3.4	17.1±0.3	17.0±0.2	37.7±0.3	28.3±2.0
Sample 2	25.5	17.1	17.3	32.3	26.9
	25.7	16.8	17.3	35.5	27.9
	23.3	17.2	17.5	32.4	28.4
Average±SD	24.8±1.3	17.0±0.2	17.3±0.1	33.4±1.8	27.7±0.8
Sample 3	23.9	18.7	17.0	35.4	26.2
	24.7	17.2	17.3	32.1	28.5
	24.8	15.1	17.5	36.1	28.9
Average±SD	24.5±0.5	17.0±1.8	17.3±0.3	34.5±2.1	27.9±1.5
Sample 4	23.8	18.7	16.4	39.1	26.9
	25.0	17.9	16.5	38.0	27.3
	23.9	17.2	15.7	34.4	29.0
Average±SD	24.2±0.7	17.9±0.8	16.2±0.4	37.2±2.5	27.7±1.1
Sample 5	23.4	18.6	15.2	34.3	24.6
	22.7	17.3	16.4	34.0	26.7
	23.6	15.0	15.3	33.9	27.9
Average±SD	23.2±0.5	17.0±1.8	15.6±0.7	34.1±0.2	26.4±1.7
Overall mean	23.9	17.2	16.7	35.2	27.6
C.V. (%)	6.95	6.34	4.61	6.45	4.82

Table 19. Heme iron (μg/g fresh weight) in beef liver and catfish mixture determined by the Hornsey method.

	Beef liver % / Catfish %				
	100/0	75/25	50/50	25/75	0/100
Sample 1	42.2	32.5	21.5	15.7	2.7
	48.1	34.0	21.7	14.9	2.7
	43.9	34.6	23.7	14.8	3.1
Average±SD	44.7±3.0	33.7±1.1	22.3±1.2	15.1±0.5	2.8±0.2
Sample 2	43.3	32.5	22.1	14.9	3.1
	43.7	32.4	22.7	13.8	3.2
	42.9	33.4	22.5	13.8	3.7
Average±SD	43.3±0.4	32.8±0.6	22.4±0.3	14.2±0.6	3.3±0.3
Sample 3	50.0	34.8	21.3	14.1	2.9
	51.2	34.4	21.9	14.4	3.2
	50.9	34.8	22.4	13.9	2.7
Average±SD	50.7±0.6	34.7±0.2	21.9±0.6	14.1±0.3	2.9±0.3
Overall mean	46.2	33.7	22.2	14.5	3.0
C.V. (%)	8.09	3.06	3.29	4.48	10.89

Table 20. Nonheme iron (μg/g fresh weight) in beef liver and catfish mixture determined by the modified Schricker method.

	Beef liver % / Catfish %				
	100/0	75/25	50/50	25/75	0/100
Sample 1	50.3	36.7	24.8	17.2	7.5
	46.5	35.4	24.9	17.3	7.5
	47.8	35.6	25.0	17.4	7.5
Average±SD	48.2±1.9	35.9±0.7	24.9±0.1	17.3±0.1	7.5±0.0
Sample 2	49.0	35.5	24.8	15.8	7.5
	48.4	34.6	25.1	16.0	7.3
	47.4	34.9	24.6	16.2	7.0
Average±SD	48.3±0.8	35.0±0.5	24.8±0.3	16.0±0.2	7.3±0.3
Sample 3	40.3	33.0	18.1	10.8	7.1
	37.0	32.8	18.7	12.7	7.0
	39.9	33.7	19.1	12.8	6.7
Average±SD	39.1±1.8	33.2±0.5	18.6±0.5	12.1±1.1	6.9±0.2
Overall mean	45.2	34.7	22.8	15.1	7.2
C.V. (%)	10.60	3.75	13.73	15.95	4.17

Table 21. Nonheme iron (μg/g fresh weight) in beef liver and catfish mixture determined by the sodium pyrophosphate method.

	Beef liver % / Catfish %				
	100/0	75/25	50/50	25/75	0/100
Sample 1	46.1	37.8	23.6	17.7	5.8
	41.2	36.6	26.1	18.2	6.0
	46.3	36.8	23.5	17.7	7.1
Average±SD	44.5±2.9	37.1±0.6	24.4±1.5	17.9±0.3	6.3±0.7
Sample 2	52.6	38.8	24.4	18.1	7.1
	48.8	39.4	27.4	19.4	7.9
	51.3	40.6	27.4	18.9	7.1
Average±SD	50.9±1.9	39.6±0.9	26.4±1.7	18.8±0.7	7.3±0.4
Sample 3	39.0	33.4	23.4	15.6	7.0
	39.2	32.3	22.4	15.6	7.1
	36.1	33.2	22.5	16.6	6.9
Average±SD	38.1±1.7	33.0±0.6	22.8±0.5	15.9±0.6	7.0±0.1
Overall mean	44.5	36.5	24.5	17.5	6.9
C.V. (%)	13.21	8.13	8.00	7.75	9.03

Table 22. Moisture analysis (%).

	Baked ground beef						
	Rare	Medium	Well-done	Beef	Chicken	Beef liver	Catfish
	53.4	51.3	50.0	55.8	74.0	70.2	72.5
	52.0	51.4	49.2	56.8	74.5	70.0	72.1
	53.3	52.7	48.5	57.9	74.1	68.7	74.7
	55.8	50.0	49.7	56.5	73.0		
				55.4	77.8		
Average±SD	53.6	51.4	49.4	56.5	74.7	69.6	73.1
	1.6	1.1	0.7	1.0	1.8	0.8	1.4

Table 23. Total iron (μg/g) analysis of NIST standards using the ferrozine and bathophenanthroline, and AAS methods.

	Ferrozine	Bathophenanthroline	AAS
Wheat flour 1567			
(18.3±1.0 μg Fe/g)	16.6	19.4	12.3
Sample 1	17.3	17.6	11.8
		18.7	
Average±SD	17.0±0.5	18.6±0.9	12.1±0.4
Sample 2	18.8	18.4	14.8
	18.2	18.9	14.5
	18.1	17.6	13.7
Average±SD	18.4±0.4	18.3±0.7	14.3±0.6
Overall mean±SD	17.8±0.9	18.4±0.7	13.4±1.3
Bovine liver 1577			
(268±8 μg Fe/g)	256	255	202
Sample 1	256	270	192
	262	269	195
Average±SD	258±4	265±8	196±5
Sample 2	272		226
	267		213
	267		221
Average±SD	269±3		220±7
Sample 3	267		219
	271		223
	261		235
Average±SD	266±5		226±8
Overall mean±SD	264±6		214±15
Non-fat dry milk			
(2.1 μg Fe/g)	3.7	4.5	3.0
Sample 1	4.3	4.2	2.8
	3.9	3.9	1.1
Average±SD	4.0±0.3	4.2±0.3	2.3±1.0

REAGENTS

Hornsey method

80% acidified acetone: acetone and deionized, distilled water were mixed in a ratio of 4:1. One ml of water was replaced by concentrated HCl. The amount of water added to the reagent varied according to the moisture content of the sample.

Modified Schricker method

Acid mixture: 6 N HCl and 40% trichloroacetic acid mixed in equal volumes.

Bathophenanthroline disulfonate reagent: bathophenanthroline disulfonic acid-sodium salt (Sigma Chem. Co.), 0.162 g dissolved in 100 ml deionized water to which 2 ml of 96-99% thioglycolic acid were added.

Color reagent: deionized water, saturated sodium acetate solution, and bathophenanthroline disulfonate reagent were mixed in a ratio of 20:20:1.

Saturated sodium acetate solution: sodium acetate granules were dissolved in deionized, distilled water until granules remained undissolved.

Sodium nitrite reagent, 0.39% (w/v) was prepared fresh before use.

Standard solution: 1000 ppm iron stock solution was diluted to 0.5, 1, 2, 3, 4, 5, and 10 ppm standards with deionized water.

Sodium pyrophosphate method

Saturated sodium pyrophosphate solution: sodium pyrophosphate crystals were dissolved in deionized, distilled water until crystals remained undissolved.

Trichloroacetic acid, 10% (w/v).

Standard solution: 1000 ppm iron stock solution was diluted to 0.5, 1, 2, 3, 4, 5, and 10 ppm standards with deionized water.

Wet ashing

Concentrated nitric acid

Hydrogen peroxide, 30%: Contains 0.5 to 0.05 ppm of iron. Equal amounts of hydrogen peroxide should be added to all samples to avoid problems of iron contamination.

Ferrozine method

Ammonium hydroxide, 12 N.

Buffer solution: 10% (w/v) ammonium acetate.

Ferrozine color reagent (1 mM): 0.051 g ferrozine (Sigma Chem. Co.) was diluted to 100 ml with deionized water.

pH indicator: 0.1 M ortho-nitrophenol solution.

Reducing agent: 10% (w/v) hydroxylamine hydrochloride solution.

Standard solution: 1000 ppm iron stock solution was diluted to 0.5, 1, 2, 3, 4, 5, and 10 ppm standards with deionized water.