Evaluation of Methods Used in Meat Iron Analysis and Iron Content of Raw and Cooked Meats

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

Keywords: *Iron; analysis; meat; heme*

INTRODUCTION

Iron is a trace element of considerable concern in public health. A complete, accurate, and quantitative knowledge of the levels and forms (heme or nonheme) of iron in foods is important since the bioavailability of each type of iron differs (Monsen et al., 1978). Meat is the main source of heme iron in human diets, and meat also makes a large contribution to the nonheme iron content of human diets (Carpenter and Mahoney, 1992). Levels of total, nonheme, and heme iron are often determined in meats, but little effort has been spent on validating the methods used to analyze iron.

Our first objective was to evaluate the accuracy, specificity, and precision of several methods now used to determine total, nonheme, and heme iron content in meats. We evaluated two total iron methods, two nonheme iron methods, and one heme iron method. The total iron methods used Ferrozine or atomic absorption spectrophotometry (AAS) to detect the iron in wet ash digests. The wet ashing technique was a novel procedure developed in our laboratory specifically for use with meat. It involved first digesting most of the solids with nitric acid and then employed peroxymonosulfuric acid, a strong oxidizer, to complete the oxidation. The nonheme iron methods used Ferrozine, a ferrous iron chromogen, to detect the iron in either HCl−trichloroacetic acid (TCA) extracts or sodium pyrophosphate−TCA extracts. The heme iron method was the acidified acetone extraction of Hornsey (1956). Accuracy was evaluated by analysis of independent standards and by determining iron recovery from iron spikes added to ground beef (GB). The spikes were added to each lean, raw GB; fat, raw GB; or lean, cooked GB to test if fat level or cooking interfered with the methods. Two measures of precision were calculated to reflect the repeatability (within-trial variability) and the reproducibility (between-trial variability) of each of the methods.

Our second objective was to determine the total, nonheme, and heme iron contents of various meats before and after cooking. The methods used were those found to be the most reliable and practical on the basis of our results from objective 1. The selected methods were total iron by Ferrozine detection of the iron in wet ash digests, nonheme iron by Ferrozine detection of the iron in HCl−TCA extracts, and heme iron by acidified acetone extraction. The meats and the cooking methods were as follows: ground beef, pan fry; beef round, braise; beef loin, broil; pork loin, broil; pork fresh picnic, roast; pork ham (cured), roast; lamb chop, broil; chicken breast, broil; chicken thigh, broil; turkey (ground), pan fry.

MATERIALS AND METHODS

Preparation of Ground Beef Samples. Lean GB was prepared by first trimming round roasts of all visible fat and grinding twice through a $^1/_8$ in. plate. Fat GB was prepared by adding trimmed fat back into the lean GB as it was reground through the $^1/_8$ in. plate. Cooked, lean GB was prepared by immersing hermetically sealed polyethylene bags of lean GB into boiling water until an internal temperature of 71 °C was obtained. All samples were bagged in 3.00 mil polyethylene bags [0.75 mil of nylon laminated with 2.25 mil of 6% ethylene vinyl acetate and 94% polyethylene (Koch, Inc., Kansas City, MO)], vacuum sealed at 29−30 in. of Hg in a Vacu-fresh vacuum chamber machine (Meat Packers and Butchers Supply Co., Los Angeles, CA), and stored frozen at −18 °C until used. Fat was determined by ether extraction (AOAC, 1990) using a Goldfisch fat extractor from Labconco (Kansas City, MO). The percent fat (mean ± SD) was 3.6 ± 0.6 in lean GB, 14.8 ± 3.0 in fat GB, and 4.6 ± 0.4 in cooked, lean GB.

Procedures. *Total Iron Methods.* Meat samples (ca. 2 g) were accurately weighed into 125 mL Erlenmeyer flasks, and 15 mL of concentrated nitric acid was added. Each flask was left to predigest at room temperature for 4−6 h or overnight. The flasks were placed on a hot plate set at 100 °C until dry. Hydrogen peroxide−sulfuric acid reagent (Hatch et al., 1985), containing peroxymonosulfuric acid, was added in 1 mL aliquots to each sample until they all became clear, typically after three or four additions. The flasks were left on the hot plate until all peroxide was expelled (5−10 min) and the white vapors of sulfuric acid became evident. The clear digest was allowed to cool and quantitatively transferred to 10 mL volumetric flasks using 0.01 N HCl as the rinse. Aliquots of

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the digests were analyzed for iron using Ferrozine color reagent or AAS. The Ferrozine method was as described by Carter (1971) and Stookey (1970), except that the final mix was 1 mL of sample, 1 mL of 1% ascorbic acid, 1 mL of 20% ammonium acetate, 1 mL of 1 mM Ferrozine, and 1 mL of water. For AAS, standards were prepared in a reagent blank solution that had received the same wet ashing treatment as the samples, including equal additions of the peroxymonosulfuric acid reagent.

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

Heme Iron Method. Hemin was determined using the acidified acetone extraction of Hornsey (1956), with slight modifications. A sample of meat (ca. 5 g) was placed in a 50 mL centrifuge tube, and 20 mL of acetone and 0.5 mL of HCl were added. Water was added so that total water in the tube, both from the meat and from the added water, equaled 4.5 g. The mixture was processed for 15 s with a Kinematica polytron and filtered. The absorbance of the filtrate at 640 nm was measured, and heme iron in the sample was calculated. Water content of the meat samples was determined by drying at 105 °C for 16 h (AOAC, 1990).

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

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

RESULTS AND DISCUSSION

Evaluation of Iron Methods. *Total Iron Methods.* The total iron values determined with Ferrozine or AAS were not different from the certified values for NIST standards and were similar to the calculated value of 3.35–3.38 mg of Fe/g of hemoglobin (Table 1). Thus,

Table 1. Total Iron in Various Reference Materials and Ground Beef

	total iron (μg/g)		
ref material[a]	ref value[a]	Ferrozine[b]	AAS[b]
wheat flour (SRM 1567)	14.1 ± 0.5	13.3 ± 0.5	14.0 ± 0.9
bovine liver (SRM 1577a)	194 ± 20	197 ± 4	196 ± 9
hemoglobin	3350	3220 ± 50	3280 ± 80
lean GB		24 ± 1	24 ± 1
fat GB		23 ± 1	23 ± 1
lean, cooked GB		25 ± 1	24 ± 1

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

Table 2. Recovery of Iron from Ferric Chloride and Hemoglobin Spikes Added to Lean Ground Beef, Fat Ground Beef, and Cooked, Lean Ground Beef[a]

	% recovery				
spike and meat	total iron		nonheme iron		heme iron
	Ferrozine	AAS	HCl–TCA	Na$_4$P$_2$O$_7$	Hornsey
FeCl$_3$					
lean GB	99 ± 10	100 ± 7	105 ± 8	110 ± 10	−11 ± 12
fat GB	100 ± 6	98 ± 6	107 ± 8	108 ± 10	−5 ± 7
cooked GB	98 ± 10	100 ± 9	106 ± 7	109 ± 8	2 ± 10
all meats	99 ± 4	99 ± 3	106 ± 3	109 ± 4	−5 ± 5
hemoglobin					
lean GB	98 ± 5	96 ± 5	11 ± 7	11 ± 4	96 ± 11
fat GB	94 ± 4	92 ± 3	11 ± 11	11 ± 4	103 ± 9
cooked GB	93 ± 7	92 ± 6	11 ± 11	14 ± 5	105 ± 5
all meats	95 ± 3	93 ± 2	11 ± 4	12 ± 2	101 ± 4

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

these total iron techniques performed well on standard reference materials and showed promise for use with meat. Preliminary experiments on meat indicated our procedure had several advantages compared to other techniques (Clegg et al., 1981a,b; Friel and Ngyuen, 1986; Hill et al., 1986; Kalpalathika et al., 1991; Schricker et al., 1982) including complete digestion that required no filtering of fat or other undigested components, speed of digestion, and no requirement for special equipment, such as perchloric acid hoods. Gordon (1978) reported that Ferrozine gives 8% lower values than AAS after wet digestion of seafood using HNO$_3$–HClO$_4$. However, in our hands, Ferrozine and AAS gave similar values for total iron in the standards and in GB (Table 1). The lower detection limit of the Ferrozine assay is 0.090 μg of iron (Carter, 1970), which, for our digestion, translates into about 0.5 μg of iron/g of meat.

Total iron methods recovered all of the iron added as FeCl$_3$ spikes, but only 93–95% of the iron was recovered from Hb spikes (Table 2). Recovery was especially low from fat GB or cooked GB, indicating possible problems in these samples. Since the iron values determined using Ferrozine or AAS detection were similar, the low recovery of heme iron was probably due to incomplete digestion. Fat is notoriously hard to digest, and cooking may form compounds that are also hard to digest. The repeatability and reproducibility using Ferrozine or AAS iron detection were similar (Table 3). However, these experiments were performed by personnel with significant experience with AAS. It was our experience that the Ferrozine method is a more rugged method than AAS and less susceptible to variability from operator

Table 3. Precision of the Iron Methods

method	RSD (%) for repeatability	RSD (%) for reproducibility
total iron		
Ferrozine	3.8	2.5
AAS	3.6	3.5
nonheme iron		
HCl−TCA	8.0	22
pyrophosphate	5.7	14
heme		
Hornsey	2.1	2.7

error and matrix effects. Additionally, the Ferrozine method is quickly adaptable to any laboratory with a spectrophotometer.

Nonheme Iron Methods. The nonheme methods recovered 106−109% of the iron in $FeCl_3$ spikes and recovered 11−12% of the iron from Hb spikes (Table 2). The reason for the greater than 100% recovery of nonheme iron is unclear, whereas the recovery of some heme iron indicated that a substantial amount of heme was degraded into porphyrin and ionic iron during the extraction procedures (Ahn et al., 1993). Among the iron methods tested, the nonheme methods showed the most variability, with the HCl−TCA procedure somewhat more variable than the pyrophosphate−TCA extraction (Table 3). The HCl−TCA and sodium pyrophosphate−TCA extraction methods are routinely used for nonheme iron analysis in food samples, and both methods have the advantages of being simple and requiring low operator time per sample. The major drawback of both the methods is that they are not rapid techniques. The HCl−TCA extraction was preferred because of its consistently more accurate recovery of nonheme iron.

Heme Iron Method. The Hornsey method accurately recovered the heme iron spikes (Table 2). It was also the most repeatable and most reproducible of the iron methods (Table 3). The Hornsey method is one of the most commonly used methods for quantifying heme iron in food samples. It is relatively safe, fairly simple, and rapid. Also, the sources of contamination are minimal, allowing for accurate analysis of low concentrations of heme iron.

Iron Content of Raw and Cooked Meats. The following methods were used to determine the iron content of various meats before and after cooking: total

iron by Ferrozine following wet ashing of the samples using nitric acid and peroxymonosulfuric acid, nonheme iron by Ferrozine after HCl−TCA extraction of the samples, and heme iron by the Hornsey methodology. The different meats varied ($p < 0.01$) in their content of total, nonheme, and heme iron (Table 4), and iron values were generally consistent with those we have previously reported (Buchowski et al., 1988; Carpenter and Mahoney, 1992). The total iron values did not equal the sum of nonheme iron and heme iron, probably due to a combination of overestimate of nonheme iron and underestimate of total iron (particularly its heme iron fraction) as previously discussed. The percent of heme iron to total iron varied among meats and was decreased by cooking (Table 5). The loss of heme iron with cooking has been attributed to the breakdown of heme into ionic iron and porphyrin (Ahn et al., 1993; Buchowski et al., 1988).

Significance. Reliable data are needed concerning the heme and nonheme iron content of meat. This study has established the reliability of several methods presently used to determine total, nonheme, and heme iron in meat and then used the most reliable methods to measure the iron contents of a variety of fresh and cooked meats. This has important dietary implications since heme iron is the more bioavailable iron form for humans. The Monsen model (Monsen et al., 1978) is the most commonly used model for predicting iron bioavailability in individual meals. The Monsen model uses the value of 40% for the percent heme iron to total iron in meat, fish, and poultry (MFP). However, the meats examined here contained widely differing amounts and percentages of heme iron. This suggests that the value used in the Monsen equation should not be an unvarying 40% but should be different for each particular meat consumed with a meal. The Monsen model has also been modified for use with populations (Carpenter and Mahoney, 1992). For use with this model, the average percent iron found as heme in MFP was estimated as 45%. This still appears to be a reasonable estimate based on the general pattern of MFP consumption in the United States (National Live Stock and Meat Board, 1994) and our data on the content of heme iron in various meats. However, this value may vary considerably depending on the consumption pattern of meat, fish, or poultry for a specific population. On the

Table 4. Iron Content of Different Meats on a Wet Weight Basis[a]

meat	total iron (µg/g wet)		nonheme iron (µg/g wet)		heme iron (µg/g wet)	
	raw	cooked	raw	cooked	raw	cooked
beef						
ground	22 ± 5	26 ± 3	5.5 ± 1.8	8.5 ± 1.6*[c]	20 ± 4	20 ± 2
round	21 ± 2	37 ± 4*	5.1 ± 1.6	11 ± 2*	21 ± 2	21 ± 3
loin	25 ± 4	34 ± 4*	7.1 ± 0.9	9.7 ± 1.8*	22 ± 3	26 ± 4
pork (fresh)						
loin	7.1 ± 1.3	10 ± 2*	4.3 ± 0.8	7.1 ± 1.7*	4.9 ± 1.0	2.2 ± 0.5*
picnic	12 ± 2	14 ± 2	4.2 ± 0.8	5.8 ± 0.6*	9.5 ± 1.8	9.0 ± 2.6
pork (cured)						
ham	7.3 ± 1.3	8.4 ± 1.2	3.4 ± 0.6	3.9 ± 0.5	6.1 ± 1.0	6.6 ± 1.0
lamb						
chop	16 ± 1	17 ± 1	5.2 ± 1.2	6.8 ± 0.8*	14 ± 1	14 ± 1
chicken						
breast	4.3 ± 0.4	5.4 ± 0.4*	2.9 ± 0.4	4.1 ± 0.6*	1.8 ± 0.2	1.4 ± 0.3*
thigh	8.5 ± 0.9	11 ± 1*	4.7 ± 1.0	7.6 ± 1.3*	5.1 ± 0.6	3.6 ± 0.9
turkey						
ground	13 ± 1	16 ± 1*	7.4 ± 2.0	9.9 ± 1.4*	9.4 ± 2.2	6.5 ± 1.8*
lsd[b]	2.6	3.0	1.4	1.6	2.4	2.5

[a] The reported values are mean ± SD for $n = 10$ samples. The samples were purchased at five different retail outlets on two separate days. [b] Fisher's least significant difference test for the column when F was significant at $p < 0.01$. [c] An asterisk signifies a significant difference between iron content of raw and cooked using Student's t test at $p < 0.01$.

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

	% heme iron	
meat	raw	cooked
beef		
ground	91 ± 9	79 ± 9
round	102 ± 5	55 ± 6[*c]
loin	90 ± 5	75 ± 9*
pork (fresh)		
loin	69 ± 5	22 ± 6*
picnic	81 ± 4	65 ± 10*
pork (cured)		
ham	83 ± 5	79 ± 6
lamb		
chop	92 ± 3	80 ± 5*
chicken		
breast	42 ± 5	25 ± 4*
thigh	60 ± 3	32 ± 6*
turkey		
ground	70 ± 15	40 ± 8*
lsd[b]	8	9

[a] The reported values are mean ± SD for $n = 10$ samples. The samples were purchased at five different retail outlets on two separate days. [b] Fisher's least significant difference test for the column when F was significant at $p < 0.01$. [c] An asterisk signifies a significant difference between % heme iron of raw and cooked using Student's t test at $p < 0.01$.

basis of the meats examined here, the percent MFP iron found as heme will be greater than 45% for people consuming a large proportion of their MFP as red meats but will be less than 45% for people consuming a large proportion of their MFP as fish and poultry.

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