Heme and Total Iron in Ready-to-Eat Chicken[†]

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

Keywords: Iron; analysis; poultry; meat; heme

INTRODUCTION

There are two types of dietary iron based on different mechanisms of absorption: nonheme and heme. Nonheme iron, found in plant and animal products, has a low bioavailability ranging from 2 to 20% (Monsen and Balintfy, 1982), and is influenced greatly by a variety of enhancing and inhibiting components in the diet (Bothwell et al., 1989; Carpenter and Mahoney, 1992; Monsen, 1988). Heme iron, on the other hand, is found only in meat, fish, and poultry (MFP), has a much higher bioavailability ranging from 15 to 35% (Monsen and Balintfy, 1982), and is not affected by other dietary constituents. Because of these great differences in bioavailability between nonheme and heme iron, the relative quantities of dietary nonheme and heme must be known to accurately estimate the total amount of bioavailable iron in a food.

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

MATERIALS AND METHODS

Sample Preparation. All meat samples were purchased from fast-food restaurants or grocery store delicatessens as whole muscle products in a ready-to-eat condition. Both light

(breasts) and dark (drumsticks) chicken meats were collected at four different times from six different locations for a total of 24 samples of each type of meat. Samples were deboned, where necessary, trimmed of all visible fat and connective tissue, chopped finely with a stainless steel knife, put into aluminum pouches to minimize light exposure and moisture loss, and weighed into vessels for moisture, heme, and total iron determinations. Moisture and heme iron analyses were performed on the day of meat purchase, and total iron analysis was started.

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

The resulting white ash from samples and standards was dissolved in 1 mL of 0.5 N HCl and transferred into 13×100 -mm test tubes. Standards were prepared by adding 1-mL aliquots of FeCl $_3$ (1, 2, 4, 8, and 10 μg Fe/mL) diluted from a 1000- μg Fe/mL stock solution (Fisher Scientific, Pittsburgh, PA). One milliliter of freshly prepared 1% (w/v) ascorbic acid in 0.2 N HCl was added to each test tube and mixed. After 15 min, 1 mL of 20% ammonium acetate and 1 mL of 1 mM ferrozine were added, and the volume was made to 5 mL with deionized water. The mixture was allowed to stand in the dark for 45 min, and the absorbance was measured at 562 nm against a reagent blank with a UV-2100U recording spectrophotomer (Shimadzu, Columbia, MD).

Heme Iron. Heme iron values were determined by the Hornsey method of total pigment analysis (Hornsey, 1956). Triplicate samples (10 \pm 0.1 g) of chopped meat were accurately weighed into 50-mL centrifuge tubes. To this was added about half of an acidified acetone solution containing 40 mL of acetone, 9 mL of water (taking into account the amount of moisture in the meat), and 1 mL of HCl. Each sample was homogenized for 15 s with a Kinematica polytron (Luzern, Switzerland), and the remaining acidified acetone solution was added. The samples were mixed thoroughly, the tubes were capped tightly and allowed to stand in the dark for at least 1 h before being centrifuged at 2200g for 10 min. The supernatant was then filtered (GF/A filter paper, Whatman, Maidstone, England) and the absorbance was measured at 640 nm against a reagent blank. The absorbance was multiplied by the factor 6800 and then divided by the sample

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Table 1. Heme Iron, Total Iron, Percent Heme Iron, and Moisture in Cooked Chicken Light $Meat^a$

supplier	heme iron (μg/g)	total iron (μg/g)	percent heme	moisture (%)
A	2.3	6.5	36	65
В	1.6	6.1	29	66
C	1.4	5.8	25	63
D	1.6	6.0	30	63
E	1.6	8.5	22	69
F	2.0	6.1	33	67
mean \pm SD LSD ^b	1.7 ± 0.5	6.5 ± 2.0	29 ± 11	65 ± 3

 a Values are micrograms of iron per gram of meat on a wet weight basis and are reported as means of four samples, each analyzed in triplicate. b Fisher's least significance difference (LSD) was calculated when the F value was significant at p < 0.01.

weight to give the concentration of total pigments in the meat as μg hematin/g meat. The iron content was calculated with the factor of 0.0882 μg iron/ μg hematin (Merck, 1989).

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

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

RESULTS AND DISCUSSION

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

Iron Content of Processed Poultry. Heme and total iron values for processed light chicken meat (breast) and dark chicken meat (drumstick) are given in Tables 1 and 2, respectively, and reflect the micrograms of iron per gram of tissue on a wet weight basis. There were significant differences in the values for heme

Table 2. Heme Iron, Total Iron, Percent Heme Iron, and Moisture in Cooked Chicken Dark Meat^a

supplier	heme iron (μg/g)	total iron (μg/g)	percent heme	moisture
A	5.6	13.2	43	66
В	5.5	11.3	48	66
C	3.4	11.5	32	63
D	4.6	13.7	34	64
E	5.1	11.3	45	68
F	5.6	13.8	41	64
mean \pm SD	4.9 ± 1.0	12.5 ± 1.3	40 ± 8	65 ± 3
LSD^b	1.5	1.6	10.7	

^a Values are micrograms of iron per gram of meat on a wet weight basis and are reported as means of four samples, each analyzed in triplicate. ^b Fisher's least significance difference (LSD) was calculated when the F value was significant at p < 0.01.

and total iron between sources of dark chicken meats, but not between sources of light chicken meat. There are probably greater variations in heme iron in red meats from working muscles that are well supplied with blood vessels than in light meats from sedentary muscles, which contain less myoglobin and hemoglobin.

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

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

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

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

LITERATURE CITED

- American Medical Association. *Nutrients in Processed Foods, Vitamins, Minerals*, Publishing Sciences Group: Acton, MA 1974.
- AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Ed.; Helrich, K., Ed.; AOAC: Arlington, VA, 1990; Vol. 2, Chapter 39.
- Bothwell, T. H., Baynes, R. D.; Macfarlane, B. J.; MacPhail, A. P. Nutritional iron requirements and food iron absorption *J. Intern. Med.* **1989**, *226*, 357–365.
- Björn-Rasmussen, E.; Hallberg, L.; Isaksson, B.; Arvidsson, B. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. *J. Clin. Invest.* **1974**, *53*, 247–255.
- Buchowski, M. S.; Mahoney, A. W.; Carpenter, C. E.; Cornforth, D. P. Heating and the distribution of total and heme iron between meat and broth. *J. Food Sci.* **1988**, *53*, 43–45
- Carpenter, C. E.; Mahoney, A. W. Contributions of heme and nonheme iron to human nutrition. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 333–367.
- Carpenter, C. E.; Clark, E. M. Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. J. Agric. Food Chem. 1995, 43, 1824–1827.
- Chen, C. C.; Pearson, A. M.; Gray, J. I.; Fooladi, M. H.; Ku, P. K. Some factors influencing the nonheme iron content of meat and its implications in oxidation. *J. Food Sci.* **1984**, *49*, 581–584.
- Clark, E. M. Effects of dry ashing at different temperatures, times and sample size on the quantitative recovery of minerals from corn silage and alfalfa hay. M.S. Thesis, Brigham Young University, 1976.
- Clegg, M. S.; Keen, C. L.; Lönnerdal, B.; Hurley, L. S. Influence of ashing techniques on the analysis of trace element in animal tissues: I. Wet ashing. *Biol. Trace Element Res.* **1981a**, *3*, 107–115.
- Clegg, M. S.; Keen, C. L.; Lönnerdal, B.; Hurley, L. S. Influence of ashing techniques on the analysis of trace element in biological samples: II. Dry ashing. *Biol. Trace Element Res.* **1981b**, *3*, 237–244.
- Cook, J. D.; Monsen, E. R. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. Am. J. Clin. Nutr. 1976, 29, 859– 867.

- Dickey, L. E.; Weihrauch, J. L. Composition of Foods. Fast Foods, Raw_Processed_Prepared; Agriculture Handbook 8-21, U.S. Government Printing Office: Washington, DC, 1988.
- Duewer, L. A.; Krause, K. R.; Nelson, K. E. U.S. poultry and red meat consumption, prices, spreads, and margins. USDA, ERS, Ag. Info. Bulletin 684, U.S. Government Printing Office: Washington, DC, 1993.
- Han, D.; McMillin, K. W.; Godber, J. S.; Bidner. T. D; Younathan, M. T.; Marshall, D. L.; Hart, L. T. Iron distribution in heated beef and chicken muscles. *J. Food Sci.* **1993**, *58*, 697–700.
- Hornsey, H. C. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *J. Sci. Food Agric.* **1956**, 7, 534–540.
- Hutchinson, G. I.; Thomas, D.; Truswell, A. S. Nutrient composition of Australian chicken. *Food Technol. Aust.* 1987, 39, 196–198.
- Jansuittivechakul, O.; Mahoney, A. W.; Cornforth, D. P.; Hendricks, D. G.; Sisson, D. V. 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.* **1986**, *51*, 263–267.
- Kalpalathika, P. V.; Clark, E. M.; Mahoney, A. W. Heme iron content in selected ready-to-serve beef products. *J. Agric. Food Chem.* **1991**, *39*, 1091–1093.
- Krzywicki, K. The determination of haem pigments in meat. *Food Sci.* **1982**, *7*, 29–36.
- Ladikos, D.; Wedzicha, B. L. The chemistry and stability of the haem-protein complex in relation to meat. *Food Chem.* **1988**, *29*, 143–155.
- McCance, R.; Holland, B. *McCance and Widdowson's The Composition of Foods*, 5th ed.; The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food: Cambridge, U.K., 1991.
- Merck Index, An encyclopedia of chemicals, drugs, and biologicals, 11th ed., Merck and Company: Rahway, NJ, 1989.
- Monsen, E. R. Iron nutrition and absorption: Dietary factors which impact iron bioavailability. *J. Am. Diet. Assoc.* **1988**, *88*, 786–790.
- Monsen, E. R.; Balintfy, J. L. Calculating dietary iron bioavailability: refinement and computerization. *J. Am. Diet. Assoc.* **1982**, *80*, 307–311.
- Monsen, E. R.; Hallberg, L.; Layrisse, M.; Hegsted, D. M.; Cook, J. D., Mertz, W.; Finch, C. A. Estimation of available dietary iron. *Am. J. Clin. Nutr.* **1978**, *31*, 134–141.
- Schricker, B. R.; Miller, D. D. Effects of cooking and chemical treatment on heme and nonheme iron in meat. *J. Food Sci.* **1983**, *48*, 1340–1349.
- Torrence, J. D.; Bothwell, T. H. A simple technique for measuring storage iron concentrations in formalinised liver tissue. *S. Afr. J. Med. Sci.* **1968**, *33*, 9–11.
- Warriss, P. D.; Brown, S. N.; Adams, S. J. M. Variation in haem pigment concentration and colour in meat from British pigs. *Meat Sci.* **1990**, *28*, 321–329.

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