Total and haem iron content lean meat cuts and the contribution to the diet

*Beulah Pretorius^a, Hettie C. Schönfeldt^a & Nicolette Hall^a

^aDepartment of Animal and Wildlife Science, Institute of Food Nutrition and Well-being, University of

Pretoria, Pretoria, Gauteng, South Africa.

Highlights

• Species, cut and age have an influence on percentage haem iron.

• The meats in this study contain higher percentage of haem iron than in the Monsenmodel.

• Haem iron value should not be a constant value, but different for each meat type.

• Inclusion of haem iron values into food composition tables is necessary.

Abstract – This study provides data on the total and haem iron contents in raw lean beef, chicken, lamb and pork meat samples. Total iron, expressed as mg/100g edible portion on fresh weight basis in raw lean beef (A-age), lamb, pork and chicken average 1.58, 1.64, 0.81 and 0.78 respectively. The haem iron content in beef (A-age), lamb, pork and chicken are 77%, 81%, 88% and 74% respectively of total iron. This has important dietary implications in calculating haem iron fractions of meat as this is higher than the common value used in the Monsen equation.

Key Words - total iron content, haem iron content, meat, food-based, iron intake

1 INTRODUCTION

Iron (Fe) deficiency is one of the most widely known nutritional disorders that affect an estimated two billion people worldwide. It occurs when there is a negative balance between iron requirements, absorption and losses. In developing countries iron

deficiency is caused not only by an iron-deficient diet but also by low bioavailability of iron in the diet. Pregnant women, infants, young children and adolescents have higher iron requirements and are at greater risk of developing iron deficiency (Zimmermann & Hurrell, 2007). Despite the numerous initiatives implemented to control iron deficiency the problem persists along with substantial health and economic costs.

Food-based approaches as one of the more sustain ways to combat iron deficiency towards increasing iron intake, depends on reliable and relevant data about the iron composition (content, as well as availability) of a food. There are two primary forms of iron that are found in food, namely, haem and non-haem iron. Haem iron is derived mainly from haemoglobin and myoglobin in animal tissue, and according to the accepted Monsen model, makes up about 40 % of total iron. Non-haem iron is found mostly in plant-based foods, and makes up the remaining 60 % of iron in animal products. In most countries, no reference is made to the specific type of iron found in food sources (Monsen, et al., 1978). Centre to this problem is that the single reference of total iron intake does not indicate the amount of iron that is absorbed by the body. The type of iron (haem or non-haem) differs in bio-availability. In general, the rate of non-haem iron absorption is related to its solubility in the upper part of the small intestine. The presence of soluble enhancers (ascorbic acid) and inhibitors (phytates, polyphenols and calcium) consumed during the same meal will have a significant effect on the amount of non-haem iron absorbed. Haem iron is much less affected by other dietary factors and contributes significantly to absorbable iron (Zimmermann & Hurrell, 2007; Pettit, Rowley & Brown, 2011).

To date, haem intake is usually assessed by applying a fixed factor to the total iron content of all meat items - 40% of total iron from meat (Monsen, et al., 1978; Monsen & Balintfy, 1982) - regardless of the origin of the meat. However, it is apparent from the literature that not only the absolute total iron content differs substantially between meat from different origins, but also the percentage iron from haem. To determine iron intake more accurately by using a meat-specific factor, more specific data on meat from different species and different retail cuts is necessary. No data on the haem iron content of South African meat is currently available. If these values are known it will significantly contribute towards consumer education about the role of meat in the diet of all South Africans.

This study aims to determine the total and haem iron content in South African meat (beef, lamb, pork and chicken). The haem iron content of different South African meats can be added to the National Food Composition Database to provide a more accurate reference of the amount of absorbable iron in South African foodstuffs.

2 MATERIALS AND METHODS

2.1 Sampling Procedure

Nine Bonsmara carcasses of the A age group (with no permanent incisors), AB age group (with 1 to 2 incisors), B age group (with 2 to 6 incisors) and six carcasses of the C age group (with more than 6 incisors) were directly obtained from an abattoir. The shoulder, prime-rib and rump were selected for analyses. These cuts were selected as they represent the composition of a typical South African beef carcass the best (Schönfeldt, 1998). Three samples from three similar cuts were pooled together as

composite samples (see Figure 1). All the meat samples were immediately refrigerated after purchase. Triplicate samples of raw commonly consumed meat cuts (lamb, pork and chicken) were obtained from four retail outlets (see Figure 1).

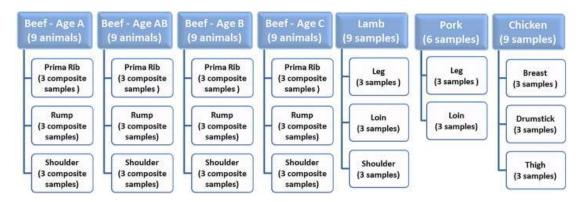


Fig. 1 : Sampling design for beef (from three age groups), lamb, pork and chicken samples.

2.2 Preparation of Samples

Raw beef, lamb and pork meat samples were de-boned and dissected into muscle, intramuscular and subcutaneous fat and bone. Chicken samples were de-boned and excess skin and fat removed. Analyses were done on muscle only. All the meat were diced, minced and freeze-dried before analyses. The samples were analysed in duplicate at Nutrilab, University of Pretoria.

2.3 Gravimetric determination of moisture

Moisture was measured in the samples by determining the loss in weight of the sample after it had been dried in an oven at 105±1°C for 16 hours. Weight loss is used to calculate dry matter content (AOAC, 2005).

2.4 Total Iron Content Analysis

The concentration of total iron in the freeze-dried meat samples was measured using the procedure described by Giron (1973), which utilizes nitric acid and perchloric acid

digestion followed by quantitation with an atomic absorption spectrophotometer (Giron, 1973). Accuracy was confirmed with NIST Standard Reference Material 1546 (meat homogenate).

2.5 Haem Iron Content Analysis

A method adapted from the Hornsey method (Hornsey, 1956; Turhan, Altunkaynak & Yazici, 2004) was developed in order to determine the haem iron content in the different animal products. Approximately 0.6 g ground desiccated meat sample was weighed into 50-ml Erlenmeyer flasks. To this, 12 ml of acid–acetone mixture was added (40 ml of acetone, 9 ml of water, and 1 ml of concentrated hydrochloric acid). The mixtures were vortex-mixed for 15 s, then, an additional 12 ml of acid–acetone mixture was added, and the samples were vortex-mixed again for 15 s. Thereafter samples were allowed to stand in the dark for 60 min and swirled by hand occasionally. The samples were filtered through glass microfiber filters (Whatman GF/A) and the absorbance measured at 640 nm against a reagent blank. The absorbance was multiplied by 6800 and then divided by the sample weight to give the concentration of total pigments in the meat as µg hematin/g meat. The iron content in hematin was considered to be 0.0882 µg Fe/µg hematin.

2.6 Statistical Analysis

Data was analyzed by Linear mixed models, using the Residual Maximum Likelihood (REML) procedure of Genstat[®]. The analysis was used to test for the effect of species and age per cut. The residuals were normal distributed and heterogeneity was accounted for (Payne, Welham & Harding, 2013). Fisher's Protected Least Significant Differences

(FPLSD) test at the 5% level was used to separate means. The data was analysed with Genstat® SoftwareTM (Payne, Murray, Harding & Baird, 2013).

3 RESULTS AND DISCUSSION

In Table 1 total, haem and percentage haem iron content in retail cuts from beef, chicken, lamb and pork meat is reported. When comparing different cuts of beef, rump had a significantly higher (p<0.001) total iron and haem iron content compared to shoulder and prime rib. However, the percentage haem iron (% HFe) between the cuts were not significantly different (p=0.937). The difference in total iron concentration between lamb loin, leg and shoulder cuts were not significant, with lamb leg and loin having a significantly higher (p<0.001) haem iron content. The % HFe in lamb shoulder is the lowest in the retail cuts from lamb. The difference between total, haem iron and percentage haem iron between pork loin and rump were not significant (p>0.05). The total iron content of chicken breast and drumsticks were reported to be significantly lower (p=0.002) than that detected in chicken thighs. The %HFe in chicken drumsticks and thighs were significantly higher than in the breasts. This correlates with results from a previous study reporting that muscle from chicken leg and thigh have a significantly higher haemoglobin and myoglobin content than chicken breasts (Kranen, Van Kuppevelt, Goedhart, Veerkamp, Lambooy, & Veerkamp, 1999).

The observed effect of cut on haem iron content can be attributed to the fact that the shoulder in beef and lamb meat and the breast in chicken meat have lesser myoglobin and haemoglobin than rump (in beef), leg (in lamb) and drumsticks and thighs (in chicken) (Jakobsen & Bertelson, 2000; Mancini & Hunt, 2005). The shoulder cut has also more connective tissue and intramuscular fat than is observed in rump and leg cuts.

In a study done by Purchas, Simcock, Knight & Wilkinson (2003) it is reported that drip from meat released during chiller storage and on soak pads contains significant quantities of iron and particularly soluble haem iron. Ageing also significantly decrease haem iron content (Ramos, Cabrera & Saadoun, 2012). The loss in haem iron in the drip and during ageing can possibly be an explanation of the significant difference in the % HFe in lamb retail cuts. The lamb meat samples were obtained from retail outlets where the researchers have no control over the storage time and aging of the meat. This is in comparison with the beef samples with identical storage and aging conditions of all the cuts of meat and no significant difference in % HFe between the different cuts was observed.

To determine the effect of slaughtering age on the total iron, haem iron and percentage haem iron content, the mean concentration for three beef cuts from four different age groups is compared in Table 2. The age of the animal had a significant effect on the total iron (p<0.001) and haem iron (p<0.001) content, with meat from age AB animals having the highest total iron content and meat from age AB and B animals having the highest haem iron content. Meat from age A beef has the lowest % HFe, but not significantly lower than meat from age AB and age C animals. Meat from age B beef has the highest % HFe, but not significantly higher than meat from age AB and age C animals. Muscles of older animals have a higher myoglobin content (Faustman, Yin & Nadeau, 1992) explaining the higher haem iron content in the meat from age AB, B and C animals.

		% Moisture	As measured		
	n		Total iron (mg/100 g)	Haem iron (mg/100 g)	 % HFe
Beef, prime rib	12	72.57ª	2.11ª	1.69ª	80
Beef, rump	12	74.45 ^b	2.45 ^b	1.95 ^b	80
Beef, shoulder	12	74.89 ^b	2.04ª	1.65ª	81
Standard error		0.22	0.05	0.03	1.21
<i>p</i> -Value		< 0.001	< 0.001	<0.001	0.937
Lamb, loin	3	73.80	1.64	1.48 ^b	90ь
lamb, leg	3	72.64	1.69	1.42ь	84 ^b
Lamb, shoulder	3	73.64	1.59	1.08^{a}	68 ^a
Standard error		0.57	0.04	0.04	2.61
<i>p</i> -Value		0.329	0.314	< 0.001	< 0.001
Pork, rump	3	72.67	0.94	0.82	88
Pork, loin	3	71.59	0.68	0.60	88
Standard error		1.33	0.08	0.08	4.19
<i>p</i> -Value		0.594	0.065	0.111	0.99
Chicken, drumsticks	3	76.69	0.75ª	0.62 ^b	82 ^b
Chicken, thighs	3	74.47	0.97 ^b	0.70 ^b	72ª
Chicken, breasts	3	75.25	0.62ª	0.42^{a}	67ª
Standard error		0.61	0.05	0.03	2.98
<i>p</i> -Value		0.072	0.002	< 0.001	0.020

Table 1 : Moisture, total iron (TFe), haem iron (HFe) (expressed as mg/100 g edible portion on fresh

 weight basis) and percentage haem iron (% HFe), in different retail cuts of raw lean beef, lamb, chicken

 and pork.

Note: Means with the same letter in a column are not significantly different.

Table 2 : Moisture, total iron (TFe), haem iron (HFe) (expressed as mg/100 g edible portion on fresh weight basis) and percentage haem iron (% HFe), in raw lean beef from three different age categories.

			As measured			"Total iron	
		%	Total iron	Haem iron	%	(Schönfeldt, Naudé, & Boshoff,	
	n	Moisture	(mg/100 g)	(mg/100 g)	HFe	2010)	
Age A	9	74.70 ^b	1.58ª	1.21ª	77ª	0.94	
Age AB	9	74.37 ^b	2.60°	2.05°	79 ^{ab}	1.08	
Age B	9	73.89 ^{ab}	2.36 ^b	2.00°	85 ^b		
Age C	9	72.92ª	2.26 ^b	1.79 ^b	79 ^{ab}	1.94	
Standard error		0.30	0.07	0.04	1.62		
<i>p</i> -Value		0.002	< 0.001	<0.001	< 0.001		

Note: Means with the same letter in a column are not significantly different. # Total iron content of meat with subcutaneous and intramuscular fat.

Total and haem iron contents in meat samples from different species (beef, lamb, pork and chicken) are presented in Table 3. A-age beef is the most commonly consumed in South Africa and therefore it was used in this comparison. Meat from the ruminant species, beef and lamb meat have significantly higher total iron (p<0.001) and haem iron (p<0.001) content with chicken and pork meat having lower values. According to (Lawrie, 1998) beef and lamb has a higher iron content relating to a higher myoglobin content present, explaining the higher haem iron values .

 Table 3 : Moisture, total iron (TFe), haem iron (HFe) (expressed as mg/100 g edible portion on fresh

 weight basis) and percentage haem iron (% HFe), in raw lean meat from different species.

		As measured				
	n	% Moisture	TFe (mg/100 g)	HFe (mg/100 g)	% HFe	
Beef (A-age)	9	74.70 ^b	1.58 ^b	1.21 ^b	77ª	
Chicken	9	75.47 ^b	0.78ª	0.58ª	74ª	
Lamb	9	72.36ª	1.64 ^b	1.32 ^b	81 ^{ab}	
Pork	6	72.13ª	0.81ª	0.71ª	88 ^b	
Standard error						
Min. rep		0.475	0.05	0.05	2.58	
Max. rep		0.388	0.04	0.04	2.11	
<i>p</i> -Value		< 0.001	< 0.001	< 0.001	0.001	

Note: Means with the same letter in a column are not significantly different.

The wide variation observed might be due to different methods used (direct haem iron determination vs determination of non-haem iron); or it might also be due to different retail cuts analysed, different age of the animals when slaughtered.

The mean total iron concentrations in muscle from the different species (beef, chicken, lamb and pork) was in the range generally reported in the literature for these meats as indicated in Table 4. In this study the total iron content for lamb, chicken and pork meat was in the generally reported range, as well as, the percentage haem iron (%HFe) in lamb meat. The % HFe for chicken and pork meat was higher than reported. The TFe in the beef samples in Table 4 were lower than the reported values in literature because it is samples from younger animals (A-age), such as in the study from (Purchas, et al., 2006). The iron values of older animals (Age AB, B and C) as reported in Table 3 are in the range compared with the reported values in Table 4. Another factor the authors observed is the lack of %moisture reported in the articles. The higher moisture content

of the samples may act as a dilutant of the nutrients and resulting in lower concentration of nutrients.

Table 4 : Moisture, total iron (TFe) and percentage haem iron (% HFe) in different meat samples as

 reported by various authors.

Mean

% Moisture TFe (mg/100 g) % HFe

Lamb, raw			
Current study	72.36	1.64	81
Schricker, Miller and Stouffer (1982)		1.64	57
Lombardi-Boccia, Martinez-Dominguez and Aguzzi (2002	2)	2.23	75
Carpenter and Clark (1995)		1.6	88
Beef, raw			
Current study	74.70	1.58	77
Schricker et al. (1982)		2.61	62
Leonhardt and Wenk (1997)		7.7	61
Lombardi-Boccia et al. (2002)		2.09	87
Carpenter and Clark (1995)		2.50	88
Kalpalathika, Clark and Mahoney (1991)	60.4	3.34	62
Purchas, Busboom, and Wilkinson (2006)		1.75	88
Pork, raw			
Current study	72.13	0.71	88
Schricker, Miller, and Stouffer (1982)		1.00	49
Leonhardt and Wenk (1997)		1.90	58
Lombardi-Boccia, Martinez-Dominguez and Aguzzi (2002	2)	0.42	62
Carpenter and Clark (1995)		0.71	69
Chicken, raw			
Current study	75.47	0.78	74
Leonhardt and Wenk (1997)		2.20	61
Lombardi-Boccia, Martinez-Dominguez and Aguzzi (2002	2)	0.59	38
Carpenter and Clark (1995)		0.64	54

Interest in haem iron intake in food intake studies has been escalating over recent years. The specific variable amount of haem iron in animal sources plays a significant role in iron absorption as a much greater proportion of haem iron is absorbed compared to nonhaem iron, even in the presence of enhancers. There are two methods of estimating haem iron intake: by using 40 % of total iron from meat (Monsen & Balintfy, 1982) or by using meat specific values. In context, a 90 g portion of beef steak (as indicated by the South African Food Based Dietary Guideline) (Schönfeldt, Pretorius & Hall, 2013) with 2.21 mg iron (analysed), will according to the Monsen model have a haem content of 0.88 mg (40 %), and a nonhaem content of 1.33 mg (60 %). With the bioavailability of haem iron being 15 to 35 %, and nonhaem iron being 2 to 20 % (Clark, Mahoney & Carpenter, 1997), the calculated amount of iron that will be absorbed will be between 0.2 and 0.6 mg, with a high probability of being in the lower range due to higher less bioavailable nonhaem iron content. Utilizing a meat specific content value of 77 % haem iron (reported in Table 3) for beef, the value of total iron that will be absorbed shifts to between 0.3 and 0.7 mg, with a higher probability to be in the higher range due to higher concentration of more bioavailable haem iron (*ie* 1.7 mg haem iron).

4 CONCLUSION

Species, cut, as well as, preparation for consumption are all factors that have an influence on the % HFe. This study provides data on total and haem iron content of different meat cuts of beef, chicken, lamb and pork. The effect of slaughtering age on total iron and haem iron in beef was also reported. As meat are normally consumed cooked, further research needs to be done to determine the iron fractions in cooked meat.

Reliable data are needed concerning the total and haem iron fractions of meat to facilitate the development of a sustainable food-based approach to combat iron deficiency. In this study the TFe and HFe contents of meat from four different species were determined. This has important dietary implications since haem iron is the more bioavailable form of iron in the human diet.

Because of the variability, the use of mean total and haem iron values for meat from different species for evaluating and predicting iron availability may be of limited value. The Monsen model (Monsen, et al., 1978) uses the value of 40% for the percentage haem iron to total iron in meat, fish and poultry. In determining the EAR's for iron, the iron bioavailability was estimated as 18% for adults (Food and Nutrition Board, Institute of Medicine, 2001). This was based on a mixed diet including all food groups. It does not consider iron from meat as an independent food group. The meats in this study contain higher percentage of haem iron. This indicated that the haem iron value used in the Monsen equation, as well as other equations, should not be a constant value, but should be different for each particular meat type consumed in the diet.

The primary need of users of food composition data bases is for data on components that affect human health. This includes the proximate nutrients, as well as specific other components, such as fatty acids and trace minerals, that are related and relevant to some distinct area of concern (Rand, Pennington, Murphy & Klensin, 1991). With varying absorption ratios of the different forms of iron a singular reference to the total iron content of a food has little value in context of nutrition. Inclusion of haem iron values

into food composition tables will provide necessary information to dieticians and nutritionists on iron availability.

5 ACKNOWLEDGEMENTS

The authors would like to thank Ms Elize Ferreira and Karin Basson form UP Nutrilab for assistance and analysis. This work is based on research support in part by the National Research Foundation of South Africa (UID: 83896) and Red meat Research and Development South Africa (RMRD SA).

6 References

AOAC. (2005). Official Methods of Analysis of the Association of Analytical Chemists, Method 935.29. 18th ed. Gaithersburg, USA: Association of Official Analytical Chemists.

Carpenter, C. E. & Clark, E. (1995). Evaluation of methods used in meat iron analysis and iron content of raw and cooked meats. *Journal of Agricultural and Food Chemistry*, Volume 43, pp. 1824-1827.

Clark, E. M., Mahoney, A. W. & Carpenter, C. E. (1997). Heme and total iron in ready to eat chickens. *Journal of Agriculture and Food Chemistry*, Volume 45, pp. 124-126.

Faustman, C., Yin, M. C. & Nadeau, D. B. (1992). Color Stability, Lipid stability, and Nutrient Composition of Red and White Veal. *Journal of Food Science*, 57(2), pp. 302-304.

Food and Nutrition Board, Institute of Medicine. (2001). *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc.* Washington DC: National Academy Press.

Giron, H. (1973). Perkin Elmer Atomic Spectrophotometer. *Atomic Absorption Newsletter*, Volume 12, p. 28.

Hornsey, H. C. (1956). The color of cooked cured pork. I.- Estimation of the nitric oxide-heme pigments. *Journal of the Science of Food and Agriculture*, Volume 7, pp. 534-540.

Jakobsen, M. & Bertelson, G. (2000). Colour stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition. *Meat Science*, Volume 54, pp. 49-57.

Kalpalathika, P. V., Clark, E. M. & Mahoney, A. W. (1991). Heme iron content in selected ready-to-serve beef products. *Journal of Agriculture and Food Chemistry*, Volume 39, pp. 1091-1093.

Kranen, R W; Van Kuppevelt, T H; Goedhart, H A; Veerkamp, C H; Lambooy, E & Veerkamp, J H. (1999). Hemoglobin and Myoglobin Content in Muscles of Broiler Chickens. *Poultry Science*, Volume 78, pp. 467-476.

Lawrie, R. A. (1998). *Lawrie's Meat Science*. 6th ed. Cambridge. England: Woodhead Publishing Limited.

Leonhardt, M. & Wenk, C. (1997). Variability of selected vitamins and trace elements of different meat cuts. *Journal of Food Composition and Analysis*, Volume 39, pp. 218-224.

Lombardi-Boccia, G., Martinez-Dominguez, B. & Aguzzi, A. (2002). Total heme and non-heme iron in raw and cooked meats. *Journal of Food Science*, 67(5), pp. 1738-1741.

Mancini, R. A. & Hunt, M. C. (2005). Current research in meat color. *Meat Science*, Volume 71, pp. 100-121.

Monsen, E. R. & Balintfy, J. L. (1982). Calculating Dietary Iron Bioavailability: Refinement and Computerization. *Journal of the American Dietetic Association*, 80(4), pp. 307-311.

Monsen, E.R., Hallberg, L., Layrisse, M., Hegsted, D.M., Cook, J.D., Mertz, W., Finch, C.A. (1978). Estimation of available dietary iron. *American Journal of Clinical Nutrition*, Volume 31, pp. 134-141.

Payne, R. W., Murray, D. A., Harding, S. A. & Baird, D. B. (2013). *Genstat*® for WindowsTM 15th Edition Introduction. UK: VSN International.

Payne, R. W., Welham, S. J. & Harding, S. A. (2013). *A guide to REML in Genstat*® *for WindowsTM 15th Edition Introduction*. UK: VSN International.

Pettit, K., Rowley, J. & Brown, N. (2011). Iron deficiency. *Paediatric and Child Health*, 21(8), pp. 339-343.

Purchas, R. W., Busboom, J. R. & Wilkinson, B. H. (2006). Changes in the forms of iron and in concentrations of taurine, carnosine, coenzyme Q10, and creatine in beef longissimus muscle with cooking and simulated stomach and duodenal digestion. *Meat Science,* Volume 74, pp. 443-449.

Purchas, R. W., Simcock, D. C., Knight, T. W. & Wilkinson, B. H. P. (2003). Variation in the form of iron in beef and lamb meat and losses of iron during cooking and storage. *International Journal of Food Science and Technology*, Volume 38, pp. 827-837.

Ramos, A., Cabrera, M. C. & Saadoun, A. (2012). Bioaccessibility of Se, Cu, Zn, Mn and Fe, and heme iron content in unaged and aged meat of Hereford and Braford steers fed pasture. *Meat Science*, Volume 91, pp. 116-124.

Rand, W. M., Pennington, J. A. T., Murphy, S. P. & Klensin, J. C. (1991). *Compiling Data for Food Composition Data Bases*. Tokyo: United Nations University Press.

Schönfeldt, H. C. (1998). *Effect of age on beef quality. PhD Thesis.* Pretoria: University of Pretoria.

Schönfeldt, H. C., Pretorius, B. & Hall, N. (2013). "Fish, chicken, lean meat and eggs can be eaten daily": a food-based dietary guideline for South Africa. *South African Journal of Clinical Nutrition*, 26(3) (Supplement), pp. S66-S76.

Schricker, B. R., Miller, D. D. & Stouffer, J. R. (1982). Measurement and content of nonheme and total iron in muscle. *Journal of Food Science*, Volume 47, pp. 740-743.

Turhan, S., Altunkaynak, T. B. & Yazici, F. (2004). A note on the total and heme iron contents of ready-to-eat doner kababs. *Meat Science*, Volume 67, pp. 191-194.

Zimmermann, M. B. & Hurrell, R. F. (2007). Nutritional iron deficiency. *Lancet*, Volume 370, pp. 511-520.