DETERMINING THE HEME IRON CONTENT OF SOUTH AFRICAN MEATS AND MEAT PRODUCTS TO INCREASE DATA ON IRON BIO-AVAILABILITY

1 INTRODUCTION

Iron deficiency is the most common and widespread nutritional disorder in the world, affecting approximately 2 billion people globally (WHO, 2009). Iron deficiency disorders mainly affect older infants, young children, women of childbearing age, pregnant and lactating women. The South African National Food Consumption Survey Fortification Baseline (SANFCS-FB-1, 2008) found that 1 out of 5 women and 1 out of 7 children in South Africa have poor iron status, making iron deficiency a national public health concern.

Iron deficiency is defined as a negative balance between the physiological needs of an individual and the amount of iron absorbed from the diet (Zimmermann & Hurrell, 2007). Iron is an element found in nature that is needed by the body for several functions such as the synthesis of certain enzymes, myogolobin and hemoglobin (Turhan, Altunkaynak & Yazici, 2004). Hemoglobin is the oxygen-carrying protein found in red blood cells that plays an important role in transferring oxygen from the lungs to other bodily tissues (Thompson & Manore, 2005). Additional to oxygen transport from the lungs, iron is also needed for the production of myoglobin. Mygolobin transports and stores oxygen within skeletal muscles and releases oxygen during muscle contractions (Whitney & Rolfes, 2005). Other needs for iron by the body are electron transport during energy metabolism as well as forming part of anti-oxidant enzymes (Yip in Bowman & Russell, 2001).

Young children with iron deficiencies suffer from decreased cognitive performance, decreased motor activity and social inattention, resulting in reduced school performance (Grantham-McGregor & Ani, 2001). Adults with iron deficiencies suffer from lowered physical activity, fatigue and reduced work performance, while pregnant women with negative iron status are at risk for preterm labour, low birth weight babies and infant and maternal mortality (Brabin, Hakimi & Pelletier, 2001; Brabin, Premji & Verhoeff, 2001; Yip in Bowman & Russell, 2001). The implications of inattention in school children and decreased work capacity in adults causes serious consequences on the development of a nation, both economically and socially. Horton and Ross (2003) estimated the annual cost caused by lowered physical and cognitive activity due to iron deficiency to be $16,78 per capita (R167,80) in developing countries. South Africa has approximately 1.2 million people that

are anaemic, this means that on an annual basis an estimation of R201 million is lost due to iron deficiency.

To overcome iron deficiency, the intake of iron either from supplements or dietary sources must meet the bodily needs. Dietary sources of iron include meat and meat products and cereal or grain-based (plant-based) products. Iron exists as two chemical forms in food either being heme iron or non-heme iron. It is generally accepted that heme iron makes up about 60% of the iron in animal-based food products while non-heme iron is the main form of iron in plant-based foods and the remaining portion of animal products (40%) (Monsen, Hallberg, Layrisse, Hegsted, Cook, Mertz & Finch, 1978). The absorption mechanisms of the two forms of iron differ and therefore affect their bio-availability. Heme iron is more bio-available for absorption, as between 15-35% is absorbed, while only about 10% of non-heme iron is absorbed from the diet (Hurrell, 2002; Zimmermann & Hurell, 2007).

Non-heme iron makes up 85% of a general diet and yet the bio-availability of non-heme iron is much lower (10%) in comparison to heme iron (15-35%) (Mckie, Marciani, Rolfs, Brennan, Wehr, Barrow, Miret, Bomford, Peters, Farzaneh, Hediger, Hentze & Simpson, 2002). The absorption of non-heme iron is affected by several factors such as the iron content of the diet, amount of storage iron in the body, enhancers and inhibitors (Thompson & Manore, 2005). The presence of iron inhibitors such as phytates, polyphenols and calcium lowers the absorption of non-heme iron, while the presence of enhancers such as ascorbic acid (vitamin C) and meat products eaten in a meal enhances the absorption of non-heme iron (Yip in Bowman & Russell, 2001; Hurrell, 2002; Zimmermann, *et al.*, 2005).

The traditional South African diet of the rural population comprises of maize meal or bread (cereal-based staple food) with a portion of vegetables and occasionally meat (Steyn & Temple, 2008). Sometimes a portion of offal products from beef, lamb/mutton, pork and chicken is part of a meal (SANFCS – South African National Food Consumption Survey, 2000). Offal is defined as the parts of animals that are left over after the carcass has been cut up, including tripe, liver, kidneys, brains, intestines, tongues, tails, head and feet (Britannica Encyclopaedia, 2009). Subba (2002) found that the iron content of offal to be high, however, there is no breakdown into the specific heme or non-heme fractions. There are no values available for the iron content of South African offal products.

The iron content of the often consumed green leafy vegetables are high, but the iron is in the less bio-available non-heme form and the presence of inhibitors such as phytates and polyphenols reduce the bio-availability even further. Meat products enhance the bio-availability of non-heme iron, therefore the addition of offal or meat high in heme iron to a

meal of green leafy vegetables and maize meal or bread will significantly increase the total percentage of iron that is absorbed. To maximise the iron absorption of iron from the diet, the right type of iron and the best possible combination of foods should be consumed.

This study aims at developing a method to determine the specific forms of iron in South African meats and offal products. After the development of the method, the heme iron content of South African meats will be determined and compared. Ample evidence suggests that although it is generally accepted that 40% of iron in all animal products is heme iron, significant differences exist in heme content of meats from different species and even between cuts within the same species. The heme iron content of different South African meats should be determined and added to the National Food Composition Database provide a better view of the amount of absorbable iron.

2 RESEARCH PROBLEM

In South Africa, as in most countries, no reference is made to the specific type of iron found in food sources. 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. As mentioned before, the type of iron (heme or non-heme) differs in bio-availability. Animal foods are considered to be good sources of the more bio-available heme iron. Monsen *et al.*, (1978) suggest that 40% of all animal iron is heme-iron, while recent literature indicates significant differences between species and even between retail cuts within the same species.

As more and more original South African values for meats become available, values of iron content differs. Table 1 summarises the iron content of different meats and other iron-rich foods in South Africa.

Table 1: Iron Content of South African Foods

Type of Food	Raw		Cooked			
		Reference	Old	Reference	New	Reference
Grilled Lamb Loin, fat trimmed	3.2	Schönfeldt *et al.*, 2007	2.0	Watt *et al*., 1975	3.3	Schönfeldt *et al.*, 2007
Grilled Mutton Loin, fat trimmed	2.8	Schönfeldt *et al.*, 2007	1.8	USDA, 1998	3.3	Schönfeldt *et al.*, 2007
Grilled Beef Fillet, fat trimmed	0.70	Schönfeldt *et al.*, 1996	3.3	Schönfeldt *et al.*, 1996	3.3	Schönfeldt *et al.*, 1996
Grilled Pork Loin, fat trimmed	1.2	Van Heerden, 2008	0.90	Watt *et al*., 1975	0.90	Watt *et al*., 1975
Roasted Dark Chicken without Skin	1.2	Schönfeldt *et al.*, 1998	0.90	SADoH, 1998	0.94	Schönfeldt *et al.*, 1998
Roasted White Chicken without Skin	1.1	Schönfeldt *et al.*, 1998	0.70	SADoH, 1998	0.70	Schönfeldt *et al.*, 1998
Imifino (Marogo) (Wild Spinach)	11.5	SADoH, 1998	3.6[4]	USDA, 1998	*	*
Amaranth	4.8	SADoH, 1998	4.6[9]	SADoH, 1998	*	*
Pumpkin Leaves	3.2	USDA, 1998	11.5[8]	SADoH, 1998	*	*
Brain, sheep/lamb, braised	*	*	1.7	USDA, 1998	*	*
Giblets, chicken, simmered	*	*	6.4	USDA, 1998	*	*
Heart, beef, simmered	*	*	7.5	USDA, 1998	*	*
Heart, sheep/lamb, braised	*	*	5.5	USDA, 1998	*	*
Kidney, beef, simmered	7.4[4]	USDA, 1998	7.3	USDA, 1998	*	*
Kidney, sheep/lamb, braised	6.4[4]	USDA, 1998	12.4	USDA, 1998	*	*
Liver, beef, fried	6.8[4]	USDA, 1998	6.3	USDA, 1998	*	*
Liver, chicken, simmered	*	*	8.5	USDA, 1998	*	*
Liver, sheep/lamb, fried	7.4[4]	USDA, 1998	10.2	USDA, 1998	*	*
Lung, beef, braised	*	*	5.4	USDA, 1998	*	*
Lung, sheep/lamb, braised	*	*	4.6	USDA, 1998	*	*
Spleen, beef, braised	*	*	39.4	USDA, 1998	*	*
Spleen, sheep/lamb, braised	*	*	38.7	USDA, 1998	*	*
Tripe, beef	2.0	USDA, 1998	*		*	*
Tongue, beef, simmered	*	*	3.4	USDA, 1998	*	*
Tongue, sheep/lamb, braised	*	*	2.6	USDA, 1998	*	*

* Values not available

Table 1 indicates that both cooked beef and lamb/mutton have higher iron values than cooked pork and chicken. The raw values for South African lamb/mutton iron values are higher than that of the beef. The dark meat of chicken has higher iron content than the white portion of chicken although the overall iron content is still lower than red meat. It can be seen from Table 1 that the iron content in chicken and red meats decrease after cooking or heat treatment. The iron content of the beef and lamb/mutton vary greatly from previous data as some of the values from the South African MRC Food Composition table are derived from values from the United States. These discrepancies in the red meat data emphasize the need to determine the most recent South African iron values for red meats. The traditional South African green leafy vegetables also have high iron content, but the form of iron is the less available non-heme iron. Offal products eaten by South Africans have very high iron values in both cooked and raw, compared to the meat and vegetables but these values are based on the United States Department of Agriculture (USDA) food composition database. There are no iron values for South African offal products and this need must be addressed.

Animal foods are considered to be good sources of the more bio-available heme iron. Monsen *et al*., (1978) suggest that 40% of all animal iron is heme-iron, while recent literature indicates significant differences between species and even between retail cuts within the same species. Red meats such as beef were recorded to be higher in bio-available heme iron (58.63%), than chicken (31.12%) and pork (36.61%) (Hallberg & Hulthen, 2000; Kongkachuichai *et al*., 2002; Lombardi-Boccia *et al*., 2002; Kalpalathika *et al*., 1991; Turhan *et al*., 2004; Purchas *et al.,* 2006 & Clark *et al*., 1997). These values are presented in table 2.

Table 2: The Heme Iron Percentages of Cooked Beef, Pork and Chicken as per Various Authors

Reference	**Country**	**Beef**	**Pork**	**Chicken**
		% Heme	**% Heme**	**% Heme**
Hallberg & Hulthen, 2000	Sweden	49.00	23.00	—
Kongkachuichai *et al*., 2002	Thailand	45.00	42.95	22.90
Lombardi-Boccia *et al*., 2002	Italy	77.25	57.50	26.35
Kalpalathika *et al*., 1991	Utah	58.53	23.00	37.50
Turhan *et al*., 2004	Turkey	46.40	—	34.36
Purchas *et al.,* 2006	New Zealand	75.60	—	—
Clark *et al*., 1997	Utah	—	—	34.50
Average		**58.63**	**36.61**	**31.12**

Apart from mutton and beef having significantly higher iron values than chicken or pork (Table 1), the bio-available heme iron in beef is also higher (Table 2). This suggests that red meats not only contributes to higher iron content in a meal, but also provides higher percentages of the more bio-

available heme iron. No data on the heme iron content of lamb/mutton is available in South Africa or globally. There is also a lack of data on the iron content and form breakdown of South African offal products. Thus the aim of this project is to develop a method to determine heme iron content of South African meats and meat products to substantiate the need to increase consumption of red meats and other meat products to alleviate iron deficiency.

3 RATIONALE

The SANFCS-FB-1 (2005) suggests that the iron status of South Africans did not improve after the implementation of the National Fortification Programme, highlighting the need to develop a method to determine the heme and non-heme iron content of South African meat and meat products. With the data available on the heme iron content of South African meat and meat products, the knowledge to promote the inclusion of eating a portion of red meat/offal products with the traditional maize meal and green leafy vegetables can be implemented to decrease the prevalence of iron deficiency in South Africa. Through consumer education about eating the right types of iron and correctly combining foods, iron deficiency can be combated.

4 LITERATURE RESEARCH

4.1 The Impact of Iron Deficiencies

The statistics of iron deficiencies worldwide suggest that it is the cause of approximately 1 million deaths in 2000 (WHO, 2002). Not only does iron deficiency cause deaths worldwide, iron deficiency is a burden of disease that has a negative impact on the social and economic growth of a country. The importance of iron in our diet as described by Thompson and Manore (2005) is to allow the functioning of certain enzymes and more importantly, the transport of oxygen around the body. Hemoglobin is quadra-poplypeptide chain that consists of 4 iron-containing heme groups. Iron binds with and releases oxygen from these heme groups to allow the transport of oxygen around the body (Yip in Bowman & Russell, 2001). Another protein in the body that requires iron and transports oxygen is myoglobin. This protein is responsible for the release and storage of oxygen in muscle cells during muscle contraction (exercise) (Whitney & Rolfes, 2005). Enzymes such as the cytochromes require iron as co-enzymes. Cytochromes are electron carriers within metabolic pathways that produce energy (Thompson & Manore, 2005). Iron is also required by the enzymes involved in anti-oxidant pathways, thus iron is an important component of the metabolic functions within our body systems.

Iron deficiency is observed when then supply of iron is not sufficient to meet the functional needs of the body after storage iron has depleted (Thompson & Manore, 2005). Iron deficiency typically affects infants, young children, adolescent girls, pregnant and lactating women (Zimmermann &

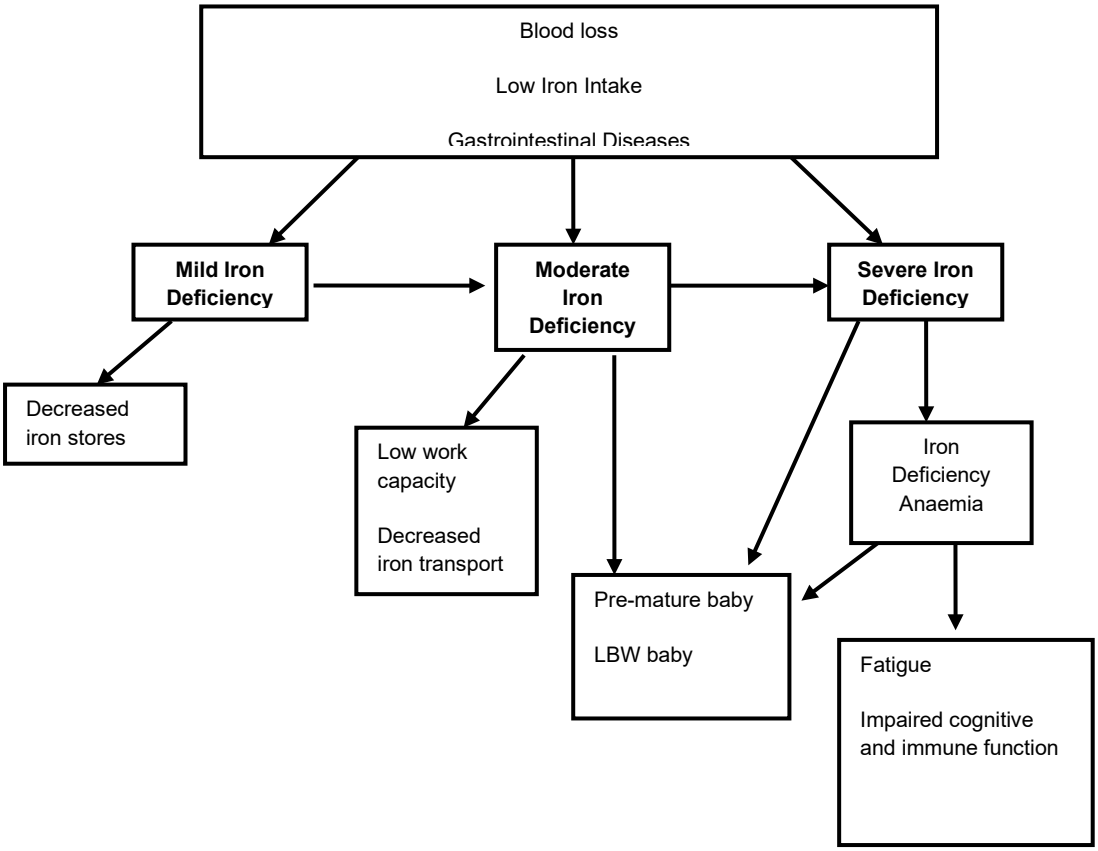
Hurrell, 2007). Inadequate dietary intake of bio-available iron is the typical cause of iron deficiency. Loss of iron (blood loss) through menstruation in women and infection such as gastrointestinal parasites (whip and hookworms) also deplete the iron stores (Krishnaswamy in Elmadfa, 2001). Adolescents and especially teenage girls require more iron in the diet due to rapid growth and menstrual blood loss, respectively (Yip in Bowman & Russell, 2001). Infants and young children undergo rapid growth and also require more iron. If insufficient iron is provided in the diet, the iron stores from birth are depleted and iron deficiency is seen (Yip in Bowman & Russell, 2001; Zimmermann & Hurrell, 2007). Pregnant and lactating mothers need to increase their iron intake to have a sufficient store of iron to support the growing foetus and breastfeeding of their new born, providing the infant with sufficient iron. Supplementation in addition to an iron-rich diet is usually recommended to ensure adequate iron intake of pregnant and lactating mothers (Zimmermann & Hurrell, 2007).

Thompson & Manore (2005) propose that iron deficiency occurs in 3 stages. The first stage of iron deficiency starts with a decrease in iron stores. At this stage of the iron deficiency, there are no physical symptoms visible because the hemoglobin levels are not affected yet. The second stage of iron deficiency results in a decrease of transport of iron around the body. The protein responsible for iron transport, transferrin reduces in number. The physical manifestation of reduced transferrin is reduced work capacity. The third and final stage of iron deficiency is iron deficiency anaemia (IDA) - the manifestation of severe of iron deficiency. During IDA, the production of normal, healthy red blood cells decrease and hemoglobin levels are inadequate to support oxygen transport. IDA can also develop during pregnancy because of increased iron needs to supply the growing foetus (Yip in Bowman & Russell, 2001). If a woman is iron-deficient during pregnancy and birth, the chances of the mother giving birth to pre-mature and low birth weight babies (LBW) are higher with risks of maternal deaths during labour (Brabin *et. al*, 2001 & Brabin *et al*., 2001). The consequences of IDA are reduced work capacity, fatigue, impaired immune and cognitive functions in both children and adults (Yip in Bowman & Russell, 2001; Rastogi & Mathers, 2002; Zimmermann & Hurell, 2007). Children with low iron stores are more prone to inattention during school, reduced cognitive and motor development (McCann & Ames, 2007), leading to adults who are potentially both cognitively and physically hindered. IDA affects approximately 1.2million South Africans, thus R201 million is lost annually due to iron deficiency causes.

Odhav, Beekrum, Akula & Baijnath (2007) observed that the consumption of nutrient-rich green leafy vegetables among the younger generation of South Africans has decreased, causing an increase in nutritional deficiencies. Steyn (2005) also observed a decrease in offal meat consumption per capita. In 2003, South Africa launched the National Food Fortification Program in hopes of reducing iron deficiency in addition to other micronutrient malnutrition. The SANFCS-FB-1

(2008) found that the iron deficiency problem still persists after the fortification programme was launched, thus the need to address iron deficiency at a national level in South Africa. Figure 1 indicates how iron deficiency leads to IDA and to other severe outcomes.

Figure 1: Schematic Diagram of Iron Deficiency Causing IDA and other severe outcomes (Adapted from Rastogi & Mathers, 2000)



4.2 Heme and Non-Heme Iron

Most literature only takes into account the total iron content of food and does not differentiate between the two chemical forms of iron: heme and non-heme iron. As discussed earlier, these two forms of iron differ significantly in their bio-availability based on different absorption mechanisms. Heme iron is mostly found in animal-based products (60%) and non-heme iron is found in the remainder of animal products (40%) and is the main constitute of iron in plant-based foods (Monsen *et al*., 1978). Heme iron is more easily absorbed by the body as compared to the absorption of non-heme iron. Heme iron has a 15-35% bio-availability, where as non-heme iron has a bio-availability of 2-20% (Clark *et al.*, 1997; Turhan *et al.*, 2004;).

The determination and differentiation between the heme and non-heme iron content in foods is important as the total amount of iron intake does not reflect the amount of iron absorbed by the

body. Meat is the main source of available heme iron yet not all South Africans can afford to include a portion of red meat in the diet. In addition to the greater bio-availability of heme iron in meat and meat products, dietary factors such as inhibitors do not influence the bio-availability of heme iron. Meat and meat products actually increase the bio-availability of non-heme iron due to the "meat protein factor effect" (MPF) (Thompson & Manore, 2005). The exact mechanism of the MPF is not certain, but evidence suggests that meat does increase the bio-availability of non-heme iron. The presence of iron inhibitors such as phytates, polyphenols and calcium lowers the absorption of non-heme iron in plant-based foods. Phytates are found in vegetables, legumes, rice and whole grains. Polyphenols are found in tea, coffee and red wine. The addition of enhancers such as ascorbic acid (vitamin C) and meat products eaten in a meal together enhances the absorption of non-heme iron (Hurrell, 2002; Yip in Bowman & Russell, 2001; Zimmermann, *et al.*, 2005).

Dietary diversification with the inclusion of red meats/offal products and green leafy vegetables in a meal increases the amount of bio-availability of both heme and non-heme iron. Nutrition education on eating a meal with the correct types of iron-rich foods in the traditional South African diet can help to alleviate the iron deficiency situation in South Africa.

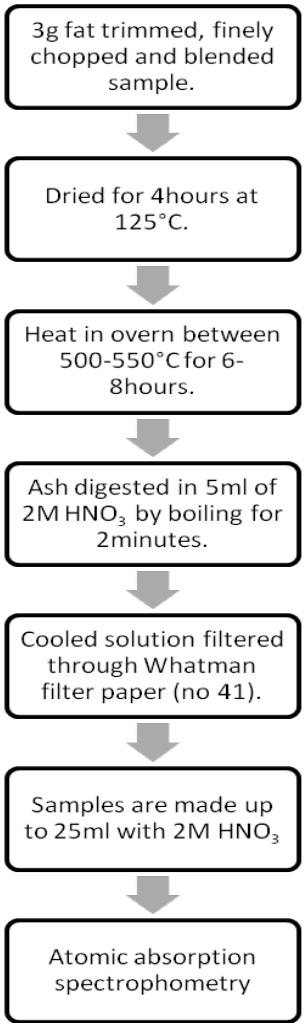
4.3 Methods Available to Determine Heme Iron Content

There is currently no official method of heme iron determination, however, there are proposed methods available that determine both the total and heme iron content of foods.

4.3.1 Total Iron Content Analysis (Atomic Absorption Spectrophotometry):

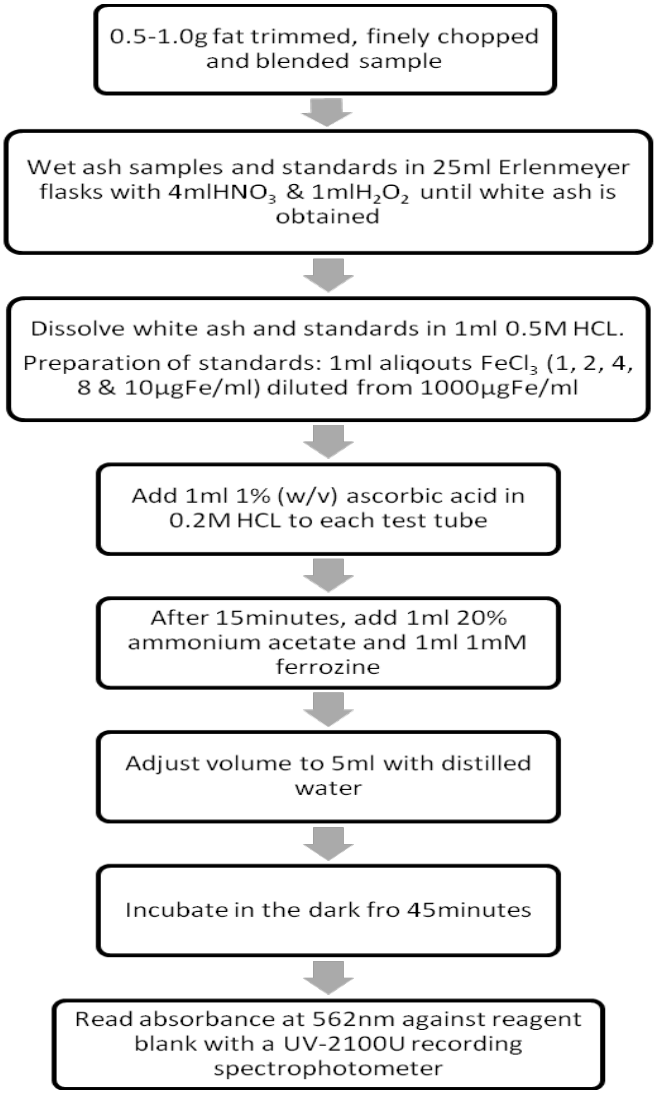
Dry Ashing: According to Turhan, *et al.*, (2004), triplicate meat samples trimmed of excess fat is ground and ashed and analysed with atomic absorption spectrophotometry at a wavelength of 248.3nm. 3g of wet-sample is dried at 125°C for 4hours and heated in an oven between 500 – 550°C for 6-8hours. The ash is then digested in 5ml of boiling 2M HNO_3 for 2minutes, then cooled to room temperature. The cooled solution is filtered through Whatman filter paper no41 and made up to 25ml with 2M HNO_3. The samples are then analysed for total iron content by spectrophometry. Figure 2 illustrates the procedure of dry ashing to determine the total iron content.

Figure 2: Schematic Diagram of Dry Ashing to Determine Total Iron Content, according to Turhan *et al.*, 2004.



Wet Ashing: Clark, Mahoney & Carpenter (1997) describe total iron content determination by deboning, trimming excess fat and connective tissues off of triplicate samples. Sample standards were obtained from the National Institute of Science and Technology (NIST). The samples and standards are then finely chopped and weighed. 0.5g – 1g samples are weighed into 25ml Erlenmeyer flasks and wet ashed first with nitric acid (4ml HNO_3) and then with hydrogen peroxide (1ml H_2O_2) at non-boiling temperatures in a microwave digestion system until a white ash is obtained. The resulting white ash from the standards and samples is dissolved in 1ml of 0.5M HCL and transferred into 13 x 100mm test tubes. Standards are prepared by adding 1ml aliquots of $FeCl_3$ (1, 2, 4, 8 and 10µgFe/ml) diluted from 1000µgFe/ml stock solution. 1ml of freshly prepared 1% (w/v) ascorbic acid in 0.2M HCL is added to each test tube and mixed. After 15minutes, 1ml of 20% ammonium acetate and 1ml of 1mM ferrozine is added. The mixture is allowed to stand in the dark for 45minutes and the absorbance is measured at 562nm against a reagent blank with a UV-2100U recording spectrophotometer. This procedure is summarised in a schematic diagram (Figure 3).

Figure 3: Schematic Diagram of Wet Ashing to Determine Total Iron Content, according to Clark *et al.*, 1997.



4.3.2 *Heme Iron Analysis (Hornsey Method, 1956):*

Method of heme iron determination described by Turhan *et al.* (2004) according to Clark *et al.* (1997), is based on acidified acetone extraction. Approximately 10g sample of meat, trimmed of excess fat is ground and weighed into 50-ml centrifuge tubes. 20ml of an acetone mixture (40ml acetone + 9ml distilled water + 1ml concentrated HCL) is added to the sample. The sample is homogenised in a blender for 30seconds or 15seconds in a Kinematica polytron. An additional 20ml of acetone mixture is added to the sample and mixed thoroughly, the tubes are capped tightly and kept in the dark for 1hour. The extract is then centrifuged at 2200g for 10minutes. The supernatant from the centrifugation is filtered through glass microfiber filters (Whatman GF/A) and the

absorbance measured at 640nm against a reagent blank. Hematin is used as a standard (Lombardi-Boccia, Marinez-Dominguez & Aguzzi, 2002). Figure 4 demonstrates the Hornsey Method.

a) Calculation of heme iron content:

The absorbance obtained is multiplied by 6800 and divided by sample weight (10g) to give the concentration of the total pigments in the meat as µg hematin/g meat.

According to Lombardi-Boccia et al., (2002), the calculation of heme iron is:

Hfe(µg/g) = hematin content (µg/g) x AW/MW

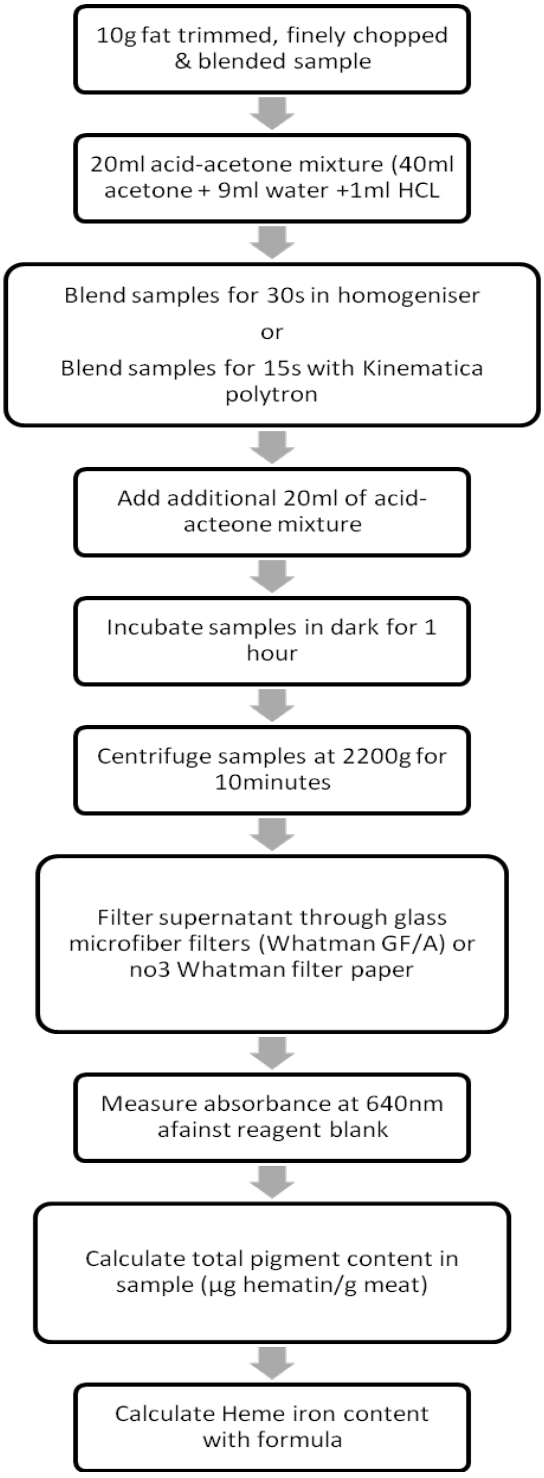
AW = Atomic Weight of iron = 55.847

MW = Molecular Weight of hematin = Iron content of hematin = 0.0882 µgFe/µg hematin.

b) Calculation of non-heme iron content:

Non-heme iron content is then calculated as the difference between the total iron content and heme iron content.

Figure 4: Schematic Diagram of Determining Heme Iron Content (Hornsey Method, 1956)



5 MODUS OPERANDI

Samples of both raw and cooked commonly consumed meat cuts (beef, mutton, lamb, pork and chicken) will be used for analysis. Convenience samples of different cuts of meat will be collected. The cuts will be vacuum packed and frozen at -20°C prior to cooking and analysis.

5.1 Preparation of Samples

Samples of raw meat will be de-boned and excess visible fat will be trimmed. Chicken samples will be de-boned and removed of excess skin and fat. The meat will then be chopped and minced.

All samples of cooked meat will first be de-boned and trimmed of excess fat then cooked according to standard cooking methods. The proposed sampling plan is indicated in Table 3. After cooking, the samples will be diced and minced. The cooking of samples will be in identical ovens at 163°C to the various internal temperatures as indicated in Table 3. The cooking losses will be measured as part of a standard procedure.

The total iron content of all the meat samples will be analysed. Methods will be identified from appropriate literature and a new method will be developed in order to determine the heme and non-heme fractions found in the different animal products.

The analysis will be performed at the Agricultural Research Centre (ARC) in Irene during 2009 and 2010.

Table 3: Experimental Design for Total and Heme Iron Analysis

Meat	Class	Cuts	Raw		Cooked Moist Heat		Cooked Dry Heat		Total	Temp	Reference
			Total Fe	Heme Fe	Total Fe	Heme Fe	Total Fe	Heme Fe			
Lamb	A2	Shoulder	3	3	3	3			12	70°C	Van Heerden & Schönfeldt, 2007
		Leg	3	3			3	3	12		
		Loin	3	3			3	3	12		
		Brain	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Kidney	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Lung	3	3	3	3			12		
		Spleen	3	3	3	3			12		
		Tongue	3	3	3	3			12		
Mutton	C2	Shoulder	3	3	3	3			12	70°C	
		Leg	3	3			3	3	12		
		Loin	3	3			3	3	12		
		Brain	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Kidney	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Lung	3	3	3	3			12		
		Spleen	3	3	3	3			12		
		Tongue	3	3	3	3			12		
Beef	A2	Loin	3	3			3	3	12	70°C	Schönfeldt, van Heerden, Visser, van Niekerk & Heinze, 1997
		Rump	3	3			3	3	12		
		Fillet	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Kidney	3	3	3	3			12		
		Liver	3	3	3	3			12		
		Lung	3	3	3	3			12		
		Spleen	3	3	3	3			12		
		Tripe	3	3	3	3			12		
		Tongue	3	3	3	3			12		
Beef	AB2	Loin	3	3			3	3	12	70°C	Schönfeldt, van Heerden, Visser, van Niekerk & Heinze, 1997
		Rump	3	3			3	3	12		
		Fillet	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Kidney	3	3	3	3			12		
		Liver	3	3	3	3			12		
		Lung	3	3	3	3			12		
		Spleen	3	3	3	3			12		
		Tripe	3	3	3	3			12		
		Tongue	3	3	3	3			12		
	C2	Loin	3	3			3	3	12		
		Rump	3	3			3	3	12		
		Fillet	3	3	3	3			12		
		Heart	3	3	3	3			12		
		Kidney	3	3	3	3			12		
		Liver	3	3	3	3			12		
		Lung	3	3	3	3			12		
		Spleen	3	3	3	3			12		
		Tripe	3	3	3	3			12		
		Tongue	3	3	3	3			12		
Chicken	White meat	Breast	3	3			3	3	12	85°C	Schönfeldt *et al*. 1998
	Dark meat	Thigh	3	3			3	3	12		
		Drumstick	3	3			3	3	12		
		Liver	3	3	3	3			12		
		Giblets	3	3	3	3			12		
Pork	P class	Loin chops	3	3			3	3	12	70°C	Am. Meat Sci. Ass 2000
		Leg roast	3	3			3	3	12		
		Loin steak	3	3			3	3	12		
TOTAL NUMBER SAMPLES			**174**	**174**	**126**	**126**	**48**	**48**	**696**		

6 REFERENCES

BRABIN B.J., HAKIMI M. & PELLETIER D., 2001, *An Analysis of Anaemia and Pregnancy-Related Maternal Mortality*, Journal of Nutrition 131: 604s-614s.

BRABIN B.J., PREMJI Z. & VERHOEFF F., 2001, *An Analysis of Anaemia and Child Mortality*, Journal of Nutrition 131: 636s-645s.

BRITANNICA ENCYCLOPAEDIA, *Offal Definition,* Web Document, http://www.britannica.com/EBchecked/topic/425640/offal. Date Accessed: 2009-01-23.

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, 45:124-126.

GRANTHAM-McGREGOR S. & ANI C., 2001, *A Review of Studies on the Effect of Iron Deficiency on Cognitive Development in Children*, Journal of Nutrition 131:649s-668s.

HALLBERG L. & HULTHEN, L. 2000. *Prediction of dietary iron absorption: An alogorithm for* calculating absorption and bioavailability of dietary iron. American Journal of Clinical Nutrition. 71:5, 1147 – 1160.

HORTON S. & ROSS J., 2003, *The Economics of Iron Deficiency,* Food Policy, 28:51-75.

HURRELL R.F., 2002, *How to Ensure Adequate Iron Absorption from Iron-Fortified Food*, NutritionReview 60: s7-s15.

HURRELL R.F., REDDY M.B., JUILLERAT M. & COOK J.D., 2006, *Meat Protein Fractions Enhance Non-heme Iron Absorption in Humans*, Journal of Nutrition, 136: 2080-2812.

KALPALATHIKA, P.V., CLARK, E.M. & MAHONEY, A.W., 199, *Heme iron content in selected Ready-to Serve beef products*. Journal of Agriculture and Food Chemistry, 39: 1091-1093.

KONGKACHUICHAI, R., NAPATTHALUNG, P. & CHAROENSIRI, R., 2002. *Heme and Nonheme Iron content of animal products commonly consumed in Thailand*. Journal of Food Composition Analysis, 15: 389-398.

KRISHNASWAMY K. In ELMADFA I., ANKLAM E & KÖNIG J.S., 2001, *Modern Aspects of Nutrition, Present Knowledge and Future Perspectives, Chapter 3.9: Anaemia – A Matter of Serious Concern and Threat to Human Development*, 17th National Congress of Nutrition, Vienna, Austria.

LOMBARDI-BOCCAI, G., MARTINEZ-DOMININGUEZ B., AGUZZI, A. & RINCON-LEON, F. 2002. *Optimization of heme iron analysis in raw and cooked red meat*. Food Chemistry, 78, 505-510.

LOMBARDI-BOCCIA G., MARTINEZ-DOMINGUEZ B. & AGUZZI A., 2002, *Total Heme and Non-Heme Iron in Raw and Cooked Meats*, Journal of Food Science, volume 67, Nr5: 1738-1741.

McCANN J. & AMES B.N., *An Overview of Evidence for a Causal Relation Between Iron Deficiency during Development and Deficits in cognitive or Behavioural Function*, American Journal of Clinical Nutrition, 85: 931-945.

McKIE A.T., MARCIANI P., ROLFS A., BRENNAN K., WEHR K., BARROW D., MIRET S., BOMFORD A.,PETERS T.J., FARZANEH F., HEDIGER M.A, HENTZE M.W & SIMPSON R.J., 2002, *A Novel Duodenal Iron-Regulated Transporter, IREG1, Implicated in the Basolateral Transfer of Iron to the Circulation*, Molecular Cell, Volume 5, Issue 2:299-309.

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 31: 34-141.

NATIONAL FOOD RESEARCH INSTITUTE, DIVISION OF FOOD CHEMISTRY, 1977, *Unpublished Report*, Pretoria: Council for Scientific and Industrial Research.

ODHAV B., BEEKRUM S., AKULA U. & BAIJNATH H., 2007, *Preliminary Assessment of Nutritional Value of Traditional Leafy Vegetables in KwaZulu-Natal, South Africa*, Journal of Food Composition and Analysis, 20: 430-435.

PURCHAS, R.W., BUSBOOM, J.R. & WILKINSON, B.H.P., 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, 74: 443-449.

RASTOGI T. & MATHERS C., 2002, *Global Burden of Iron Deficiency Anaemia in the Year 2000*, Global Burden of Disease (draft).

SANFCS-FB-1 (SOUTH AFRICAN NATIONAL FOOD CONSUMPTION SURVEY FORTIFICATION BASELINE), 2005, South African Journal of Clinical Nutrition, Volume 21, Issue 2: 245-300.

SAYED N., FRANS Y. & SCHÖNFELDT H.C., 1999, *Composition of South African Foods: Milk and Milk Products, Eggs, Meat and Meat Products*, South African Food Composition Database (SAFCoD), MRC, South Africa.

SCHÖNFELDT H.C, VAN HEERDEN S.M. & SAINSBURY J., *A Comparison of the Nutrient Quality of South African Lamb (A2) and Mutton (C2)*, Poster presented at WCAP (World Congress of Animal Production), 2007.

SCHÖNFELDT H.C., VAN HEERDEN S.M., NIEKERK J.M., VISSER R.E. & HEINZE P.H., 1998, *The Nutrient Content of South African Fresh and Frozen Chicken*, ARC-Animal & Nutrition Products Institute, Irene, South Africa.

SCHÖNFELDT H.C., VISSER R.E., NIEKERK J.M. & VAN HEERDEN S.M., 1996, *Nutritional Content of South African Beef*, ARC Meat Industry Centre, South Africa.

SOUTH AFRICAN DEPARTMENT OF HEALTH (SADoH), 1998, *Laboratory Reports and Analytical Data on South African Chicken (Fresh & Frozen)*.

SOUTH AFRICAN DEPARTMENT OF HEALTH (SADoH), 1998, *Laboratory Reports with Analytical Data on Vegetables and Fruit.*

SOUTH AFRICAN FOOD CONSUMPTION SURVEY (SANFCS), 2000, *Food Frequency Questionnaire*, Wed Document: http://www.sahealthinfo.org.za/nutrition/ffquestionare.pdf. Date Accessed: 2009-01-22.

STEYN N.P., 2005, *Chapter 4: Nutrition and Chronic Diseases of Lifestyle in South Africa*, Chronic Diseases of Lifestyle in South Africa since 1995-2005.

STEYN N.P & TEMPLE N., 2008, *Community Nutrition Textbook for South Africa: A Rights-Based Approach*, Chapter 6, Chronic Diseases of Lifestyle Unit, MRC, South Africa.

SUBBA D., 2002, *Acceptability and Nutritive Value of Keropok-like Snack Containing Meat Offal*, International Journal of Food Science and Technology, 37:681-685.

THOMPSON J. & MANORE M., 2005, *Nutrition: An Applied Approach*, Chapter 10: Nutrients Involved in Energy Metabolism and Blood Health, Pearson Benjamin Cummings.

TURHAN, S., ALTUNKAYNAK, T.B. & YAZICI, F. 2004. *A note on the total and heme iron contents of ready to-eat doner kebabs*. Meat Science, 67: 191-194.

USDA (UNITED STATES DEPARTMENT OF AGRICULTURE), 1998, *USDA Nutrient Database for Standard Reference Release 12*, Nutrient Data Laboratory Homepage: http://www.nal.usda.gov/fnic/foodcomp.

VAN HEERDEN S.M., 2008, *The Complete Guide to South African Pork*, Agricultural Research Council (ARC), Animal Production Institute, Irene, South Africa.

WATT & MERRIL, 1975, *Composition of Foods*, Agricultural Handbook no 8., Washington: United States Department of Agriculture (USDA).

WHITNEY E. & ROLFES S.R., 2005, *Understanding Nutrition*, 10th edition, Chapter 13: The Trace Minerals, Thomson Wadsworth.

WHO (World Health Organisation), 2009, *Micronutrient Deficiences Iron Deficiency Anaemia*, Electronic source: http://www.who.int/nutrition/topics/ida/en/ (Date accessed: 2008/01/14).

WHO (World Health Organisaion), 2002, *Global Chronic Diseas: 1990 – 2020*, London IBLF Dialogue.

YIP R. in BOWMAN B.A & RUSSELL R.M., 2001, *Present Knowledge in Nutrition*, 8th edition, Chapter 9: Iron, International Life Sciences Institute, ILSI Press, Washington DC, USA.

ZIMMERMAN M.B., CHAOUKI N. & HURRELL R.F., 2005, *Iron Deficiency due to Consumption of a Habitual Diet Low in Bioavailable Iron: A Longitudinal Cohort Study in Moroccan Children*, American Journal of Clinical Nutrition 81:115-121.

ZIMMERMANN M.B. & HURRELL R.F., 2007, *Nutritional Iron Deficiency*, Lancet 370: 511-520.