Comparison of Iron Intake Diets in Tanzania
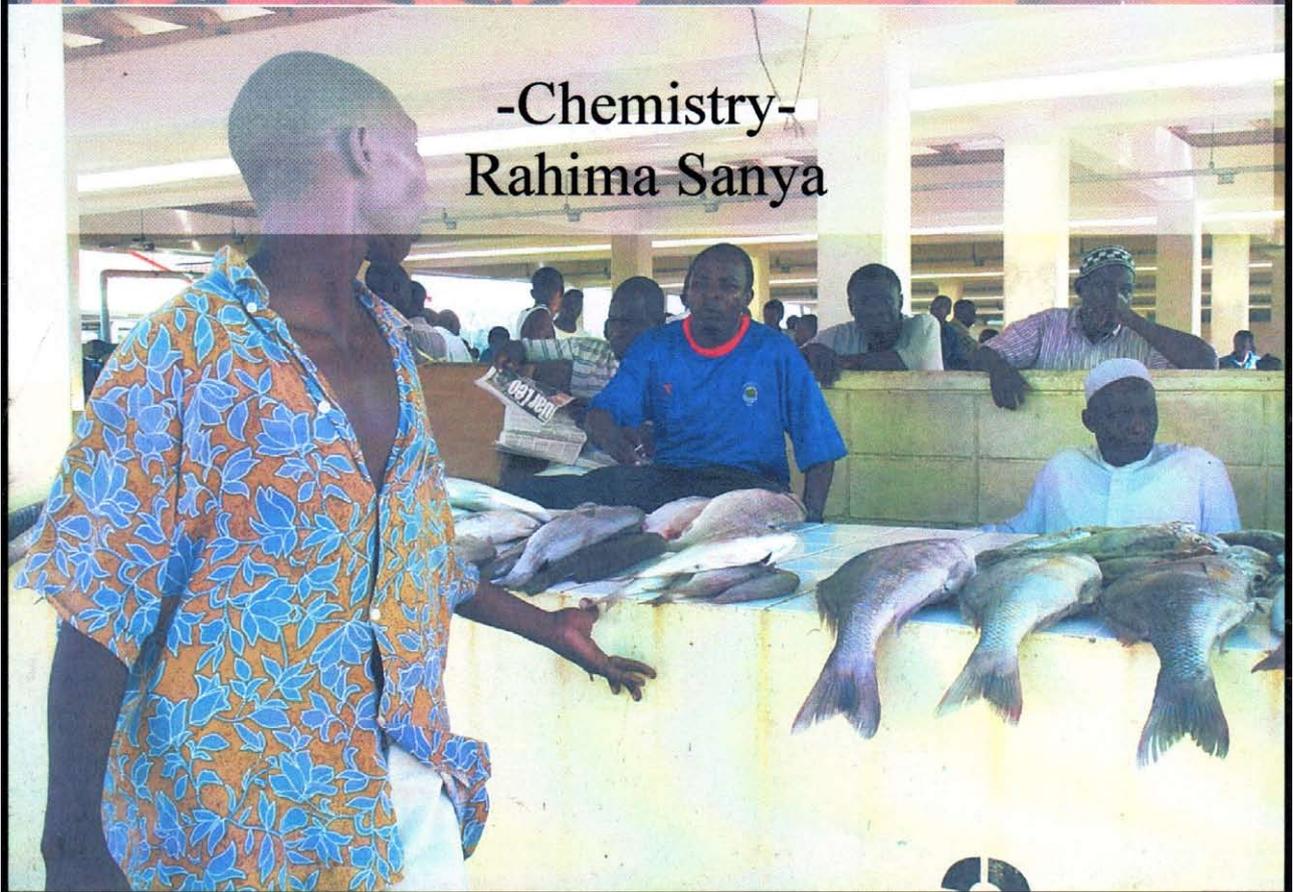
-Chemistry-
Rahima Sanya
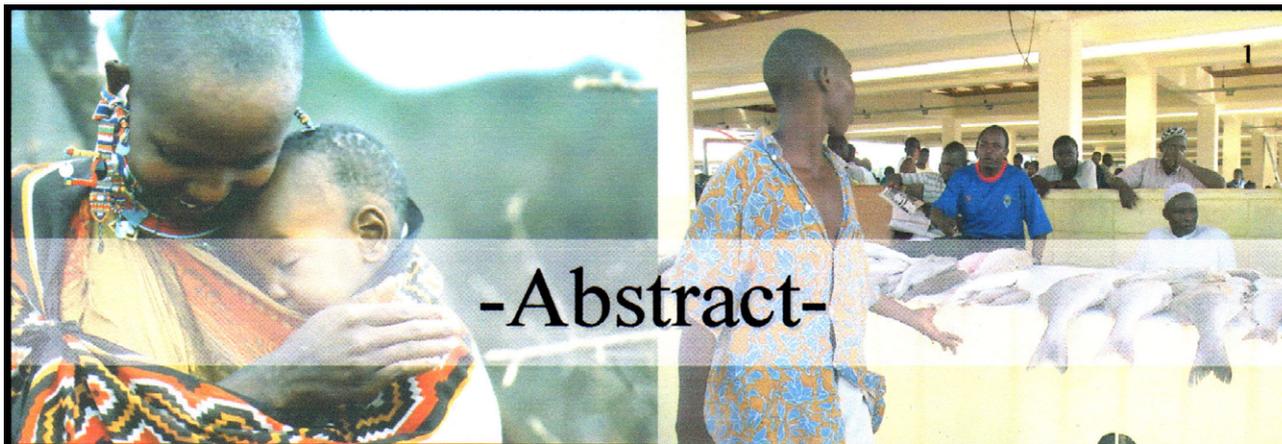
-Abstract-

Spectrophotometric determination of ratio of Iron content in diets of urbanized minimum wage workers compared with orthodox Masaai's cattle–based diet in Dar es Salaam.

Diet is largely determined by our living standards and cultures which creates barriers towards health development. Looking at two different groups in Tanzania, using a spectrophotmetric determination of the iron content in a few Tanzanian foods, this extended essay compares the ratios of iron content of diets of urbanized minimum wage worker against orthodox Masaai diet in order to understand the high rates of Iron deficiency within the Tanzanian community.

Tanzanian foods have Iron contents that still haven't been officially recorded. Using a spectrophotometer, I made a standard calibration curve, where concentration is proportional to the amount of substance absorbed (Beer's Law) of $[Fe(SCN)_6]^{3-}$ solutions. This solution consisted of Iron tetrachloride and potassium thiocyanate. The equation I derived from this curve was the basis of finding the concentrations of Iron in unknown food samples, thus the iron content in each food.

I found a ratio of 1:6 for the average amount of Iron consumed/day. The high value evident in Masaai diet reflects their ability to live in the wild away from health services. Minimum wage workers had a white flour-based diet which is a cheap product and nutritionally low in Iron. If whole grain flour could be promoted in their diets perhaps it would make it more iron rich. Low incomes play a significant role in diets and this is a large barrier to the health development of low income groups. The actual values of average Iron intake per day that I found were rather higher than what I had expected and higher than RDA value. This contradicts the iron deficiency statistics for developing countries but the ratio gives significant conclusions about Tanzanian society.
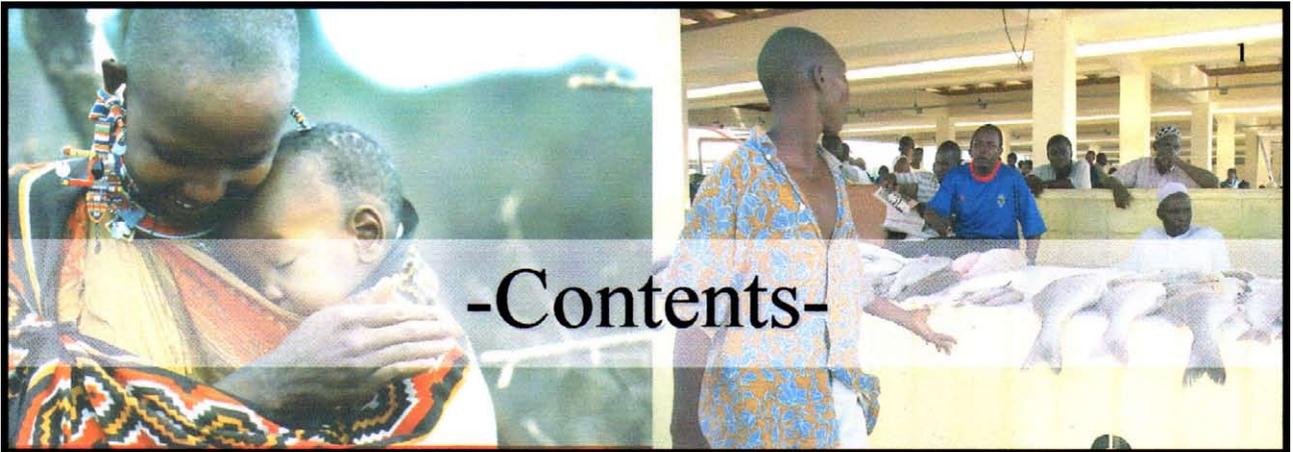
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Research question:
Spectrophotometric determination of ratio of Iron content in diets of urbanized minimum wage workers compared with orthodox Masaii's cattle–based diet in Dar es Salaam?

Iron is an essential metal ion needed in the body because it is part of the formation of hemoglobin, the red pigment in red blood cells which carries oxygen to the cells. Iron is a transition metal meaning it can be present in different oxidation states allowing it to be involved in a variety of reactions, e.g. redox reactions in the human body. Iron exists as Fe (II) or Fe (III) and is able to react and bond with nitrogenous bases in the hemoglobin and vitamin B_{12}. This is due to the "high charge densities"[8] of Iron that attracts lone pairs on the nitrogenous bases. Fe Forms the base for all covalent bonding on the "heme" group in hemoglobin.

During cellular respiration, the electron transport chain functions to produce ATP. Cytochromes on the electron transport chain produce ATP; they contain Iron. The Iron in the cytochromes changes between oxidation states Fe (II) and Fe (III), they go through a redox reaction:

$$Fe^{2+} \leftrightarrow Fe^{3+} + e^{-}$$
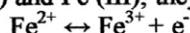
In my experiment I need to get Iron in food to undergo the same process' as existing in the human body. Fe^{2+} also exists, in the "heme" group, oxygen binds to Fe^{2+} creating oxyhemoglobin. Fe^{2+} becomes a transport system where it releases oxygen and takes carbon dioxide to the lungs.

A lack of Iron in the body causes Iron deficiency which results in anemia because the body cannot get oxygen causing e.g fatigue in the muscles. This occurs when CO binds with Fe^{2+} blocking oxygen to bind, causing the heart to pump at a greater rate to supply oxygen. Iron also helps maintain a functional immune system able to tolerate disease.

The average requirement for daily iron intake is 10 mg per day[1] and for adult woman it is 18 mg per day[1], because women need more hemoglobin due to large losses of blood from menstruation. For developing countries where food is scarce and majority of the people cannot fulfill their minimum nutritional requirements, Iron deficiency is a common problem; it "affects over 600 million people through out the world, particularly in developing countries.[4]" The purpose of this essay is to understand how both urban minimum wage Tanzanian and traditional Masaais diets compare, and how factors like cultural issues and incomes influence African diets and thus how this causes barriers to health development.

It's important to understand the cause of why diets in Tanzania vary, what barriers keep the diets either nutritionally rich or not. The Masaais live in the wild and have a strong immune system despite the distance from health care. Their cattle–based diet is a cultural factor. I predict that because of this they have a much higher iron intake than regular urban workers who face more problems due to their low income e.g. higher food prices in Dar es Salaam. To find out the diets of the different Tanzanians I interviewed two workers, working and earning an average Tanzanian minimum wage (48,000 /-). I took down what they ate for their three meals; ages were between 18 – 40 since this is an active working age. Similarly with the two masaais, I asked for their typical diet. In both groups there was a gender mix in order to make it a fair experiment. Masaais have changed their diet and due to urban influence you'll see Ugali being introduced to their diets. Urbanized Masaais askaris may alter the data however Masaais have been able to stay very cultural despite urban migration due to their orthodox ways.

METHOD:

Measuring iron content in foods:
Measuring the Iron content in food was a challenge because all the methods were complex and included concepts very hard to understand. The methods were also indirect. After researching I found the most common method used was by a spectrophotometer. The method was based on several sources but modified by me. The primary resource was a lab project from Rice University [5].

To measure the Iron content in food required 4 main processes, firstly creating solutions of potassium thiocyanate and $FeCl_3$ of known concentrations. Then making a standard calibration curve of the absorbance values of Fe^{3+} red ions against corresponding known concentrations of $[Fe(SCN)_6]^{3-}$. The most time consuming part preparing the food samples, and then reading the absorbance and calculating the iron content per gram of each sample of food that allowed me to calculate the iron content in each diets and thus compare.

1st Step: Making the necessary solutions:
Throughout the experiment 3 main solutions were needed, firstly Iron tetrachloride, $FeCl_3$ (0.0010M), potassium thiocyanate KSCN (1.5M) and hydrochloric acid HCL (2M), HCl was already a solution. Making the solutions was something I derived on my own.

Firstly the (KSCN) solution was prepared from KSCN powder into 1.5M solution. Using a $0.1dm^3$ volumetric flask; 1.5 M of KSCN of $0.1dm^3$ had to be made. This equation gives the mass of Potassium thiocyanate powder needed to make.

$$molarity = \frac{m}{M} * \frac{1}{v} \qquad \text{............1}$$

$$1.5 = \frac{m}{95.18} * \frac{1}{0.1}$$

$$m = 14.277g$$

So 14.277 g of KSCN was added to volumetric flask, and then distilled water was poured till the 100 ml mark to make KSCN solution of 1.5 M.

The other solution was iron trichloride. This was also prepared using a volumetric flask but of 1 dm^3 of $FeCl_3$ solution, (0.0010M). For this concentration of $FeCl_3$, when molar mass is 162.2 using Eq1, 0.162g of $FeCl_3$ was added to a volumetric flask and then distilled water was poured till the 1 dm^3 mark to make 0.0010 M of $FeCl_3$ solution. A larger volumetric flask was used because very little of $FeCl_3$ was needed, the larger the volume of solution, the greater the mass used and thus more accurate.

2nd Step: Making a standard calibration curve:

Five standard concentrations of $[Fe(SCN)_6]^{3-}$ (aq) were made by reacting $FeCl_3$ solution to a standard 5 ml of KSCN, using a pipette, to ensure accuracy. The reaction takes place like this:

$$Fe^{3+} (aq) + 6\ SCN^- (aq) \leftrightharpoons [Fe(SCN)_6]^{3-} (aq)$$
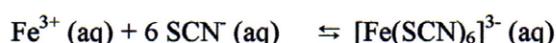
All food samples contained Fe^{3+} or Fe^{2+}, Thiocyanate plays the function of changing an iron solution with Fe Ions into a deep red solution, causing the ions turning color that can be read with the spectrophotometer. This also ensures that only the measurement of Iron is read with the

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spectrophotometer and not the chloride or other elements in food samples, because thiocyanate makes a colored analyte of Iron ions only.

Following the table below, five standard solutions were made of varied concentrations. The volume of $FeCl_3$ solution was determined like this:
Ex; for 0.00005 $moldm^{-3}$, 2.5×10^{-6} mols of $[Fe(SCN)_6]^{3-}$ are used, derived from Eq1

According to the stoichiometric ratios 1 mol of $[Fe(SCN)_6]^{3-}$ produces 1 mol of $FeCl_3$ so 2.5×10^{-6} mols of $FeCl_3$ are used to make this solution which is this volume using Eq1:
$$v = 0.00025 \ dm^3$$

Again 1 mol of $FeCl_3$ requires 6 mols of KSCN, so a constant 5 ml was used for each solution because KSCN was always in excess. The solution just needs 1.5×10^{-5} but 5 ml contains (using Eq1) .0075 mols. $FeCl_3$ becomes limiting reagent in the experiment.

After the reaction took place, the solution was diluted with HCL (2 M) to reach 50ml solution of $[Fe(SCN)_6]^{3-}$. This was done for all 5 standard solutions. (The solutions were taken from suggested concentrations from the primary source.)

Volume of $FeCl_3$ [ml] $\pm$ 0.01	Volume of KSCN [ml] $\pm$ 0.01	Concentration of $[Fe(SCN)_6]^{3-}$ [$moldm^{-3}$]	Volume of $[Fe(SCN)_6]^{3-}$ [dm^3] $\pm$ 0.06	Moles of $[Fe(SCN)_6]^{3-}$ and $FeCl_3$
2.5	5	0.00005	0.05	2.5×10^{-6}
5	5	0.00010	0.05	5×10^{-6}
7.5	5	0.00015	0.05	7.5×10^{-6}
10	5	0.00020	0.05	1×10^{-5}
12.5	5	0.00025	0.05	1.25×10^{-5}

5ml of the known concentrations were poured into cuvettes. Then was placed inside the spectrophotometer (Vernier) and the percentage transmittance was read at the blue light with a wavelength of 470nm. Because the colored analyte (Fe ions) was red a contrasting blue color light was used. The spectrophotometer gave 4 readings per second, an average transmittance was calculated. This gave me an inverse relationship for percentage transmission against concentration. Knowing that absorbance is

$$A = 2 - logT \ ^{[2]} \ \ldots\ldots\ldots 2$$

A: average absorbance
T: percentage transmitted
A graph of average absorbance over time was graphed giving a positive correlation data from which I derived a linear best fit line. This standard calibration curve is based on Beer's Law which states that "the light absorbed by a solution depends on the absorbing ability of the solute, the distance traveled by the light through the solution, and the concentration of the solution."[2] Thus concentration of solution is directly proportional to absorbance.
$$A = \varepsilon \ bC \ ^{[2]}$$

A= absorbance
ε= absorbing ability of solute
B=light path length
C=concentration

The purpose of the calibration curve is to find amount of Iron in food samples of, which I can find the absorbance. Using the found equation the absorbance of food samples were substituted in and then solved for concentration.

Step 2: Combustion of food samples and making solutions from food samples:
Firstly I researched on the diets of the Masaais and the minimum wage workers. I needed to know the diets of my tested group of people and with four interviews, rounded up diets for each person for a whole day. I asked a normal minimum wage worker, one maid, one driver, 2 guard masaais what they ate in a typical day. 2 for each situation were taken to get a more general representation of the population. Then I gathered a sample of each different food type. Age was controlled because all the individuals were in the age range of 18 to 40 years, an active age range, gender.

Taking known masses of each food sample, foods were completely combusted in crucibles over the Bunsen burner at a high heat. Occasionally I stirred them to make sure they were combusted entirely; this was so that Iron could be oxidized in the food to Fe^{2+} or Fe^{3+}, as it would be in the body. (The combustion was derived from the primary source however I assumed this could be a reason.) Then the samples were cooled off, later the samples were crushed and placed in beakers and with 5 ml of 2M hydrochloric acid to extract the iron.[5] They were left over two days; to allow the iron content in the food to be dissolved into the HCL.

Using filter paper the undissolved food samples were separated from the hydrochloric acid. 5ml of the solution was combined with 5ml of KSCN, creating a $[Fe(SCN)_6]^{3-}$ unknown concentration solution. 5ml of this solution was poured in a cuvette and transmittance was read. Using Eq2 absorbance was calculated.

Using the equation derived from the calibration curve and absorbance values, concentration of food samples could be calculated.

This was the method for finding the iron content in the food, which was used later to calculate total iron intake for the individuals in a day.

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DATA COLLECTION AND ANALYSIS:

Standard Calibration curve:
Below are the results for the transmittance for each concentration value, then absorbance was calculated using equation.

Concentration of $[Fe(SCN)_6]^{3-}$ [Moldm^{-3}] x 10^{-5}	% Transmittance ± 0.05	Absorbance Eq 2
5	24.66	0.608
10	23.97	0.620
15	23.27	0.633
20	22.88	0.641
25	22.39	0.650

This makes the standard calibration curve as seen on the next page. As the Iron content increased the solution was redder caused by greater amount of $FeCl_3$ solution, the colored analyte is due to the Fe^{3+} ions.

Using the best fit line this is the equation I derived:
$$Eq\ 3\quad A = 210[conc] + 0.5989$$
Conc. is in moldm^{-3}
A= absorbance

Food samples:
Before I could test any food samples I needed to research Since both were in convenient distance I could also observe how much they ate. This is their data, three main meals:

#1: Asha (20 years, female, house maid)
-chai and mandazi (25g)
-ugali (60g) and beans (40g)
-chai

#2: Mzee Saidi (38 years, male, driver)
-chai and mandazi (25g)
-rice (70g) and fish curry (50) (Zanzibar style)
-chai

Masaai Askaris:
#1 Yohana (around 25 years, male, askari)
- Cow Milk (500ml)
-Ugali (40g) with cow blood (250ml)
-Cow meat (100g)

#2 Sentol Mtareh (30, female, askari's wife)
- Locatario Mtindi (20g)
- Meat (50g)
- Cow blood with kiloreti, a herb (75g)

Stand Calibration Curve: Absorbance of Fe³⁺ and Fe²⁺ Ions at known concentrations of [Fe(SCN)₆]³⁻ solution.

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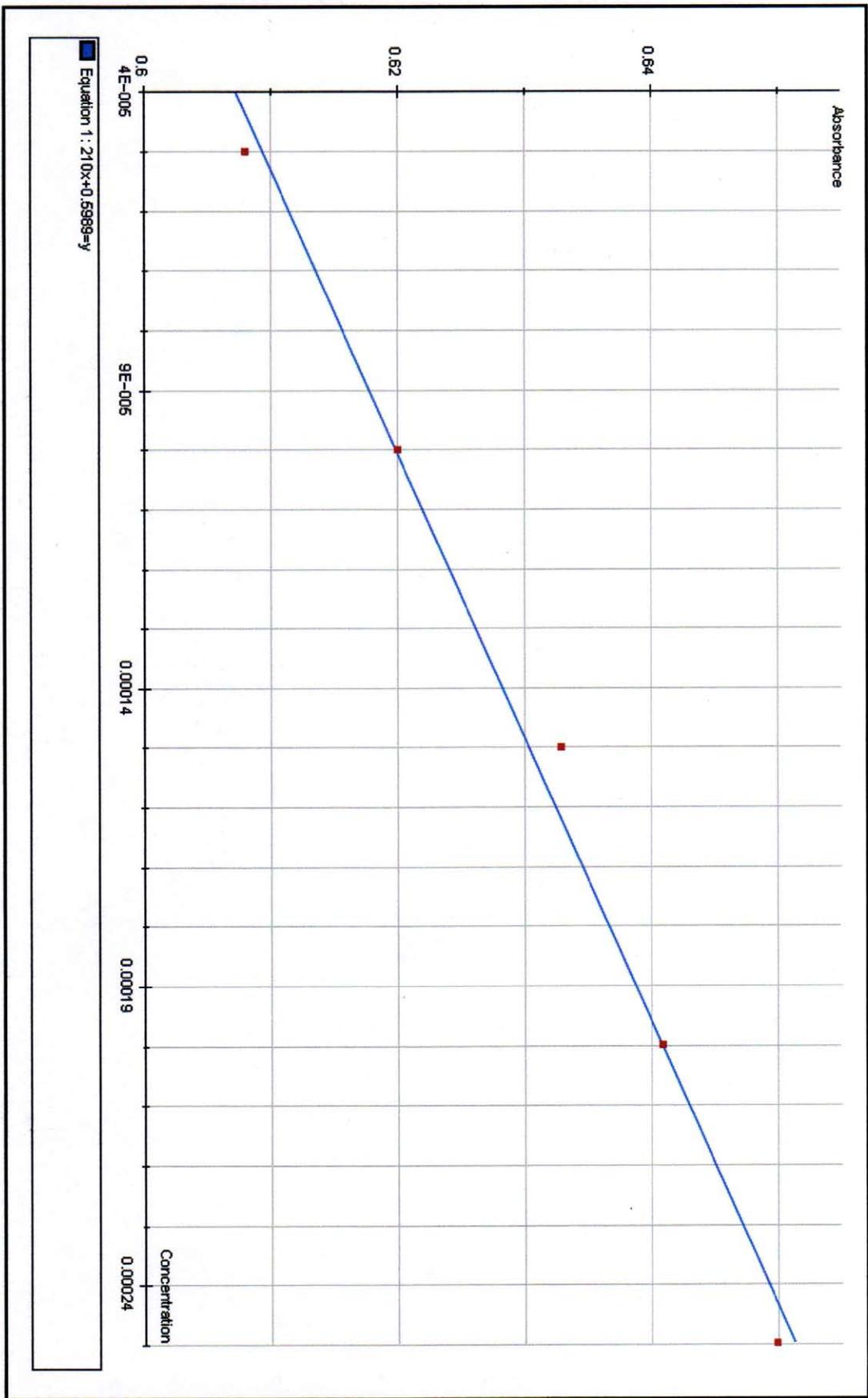
I tested the below food samples and these are the transmittance and absorbance; I didn't test for tea because I assumed tea had negligible Iron according to literature values:

Food type	Weight before Burning	Transmittance (%T)	Absorbance Eq2
Rice	1.3g	11.4019	0.943
Beans	0.4g	24.243	0.615
Meat	2.3g	8.6814	1.061
Ugali	3g	20.612	0.686
Fish	0.9g	21.997	0.658
Mandazi	5.5g	17.1984	0.765
Cow Milk	250 ml	19.346	0.713
Cow Blood with "kiloreti" (a herb)	1.9	7.9284	1.1008
"Locatria" mtindi (yoghurt)	2.3	18.239	0.739

Using Eq 3 concentration was solved for:

Food type	Weight before Burning ± 0.001g	Concentration Moldm^{-3} for 5ml x 10^{-4}
Rice	1.3g	16.4
Beans	0.4g	7.70
Meat	2.3g	22.0
Ugali	3g	4.15
Fish	0.9g	2.81
Mandazi	5.5g	7.90
Cow Milk	250 ml	0.55
Cow Blood with "kiloreti" (a herb)	1.9	23.9
"Locatria" mtindi (yoghurt)	2.3	6.71

With that I calculated the amount of iron in grams per amount of food sample using Eq 1 55.85 is the molar mass of Fe and since I had solutions of 10 ml, my volume was 0.01 dm^3.

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Food type	Mass before Burning [g] ± 0.05g	Concentration [Moldm^{-3}] x 10^{-4}	Mass of Iron [g] x 10^{-4}	Mass of Iron per unit amount of food x 10^{-4}
Rice	2.10	16.4	9.15	0.44/g
Beans	0.4g	7.70	0.43	1.07/g
Meat	2.3g	22.0	12.3	5.34/g
Ugali	3g	4.15	0.000742	0.00025/g
Fish	0.9g	2.81	1.60	1.75/g
Mandazi	5.5g	7.90	4.42	0.0803/g
Cow Milk	250 ml	0.55	3.05	12.2/L
Cow Blood with "kiloreti" (a herb)	1.9	23.9	13.4	7.02/g
"Locatria" mtindi (yoghurt)	2.3	6.71	7.02	1.62/g

After analysis of Iron content in food samples I converted the values to mg/ per unit mass, so that it's easier to deal with the figures.

Food type	Iron content (mg) per gram
Rice	0.435
Beans	0.107
Meat	0.534
Ugali	0.0000025
Fish	0.175
Mandazi	0.0803
Cow Milk	1.218/L
Cow Blood with "kiloreti" (a herb)	0.702
"Locatria" mtindi (yoghurt)	0.162

Comparing the Iron content through a bar chart:
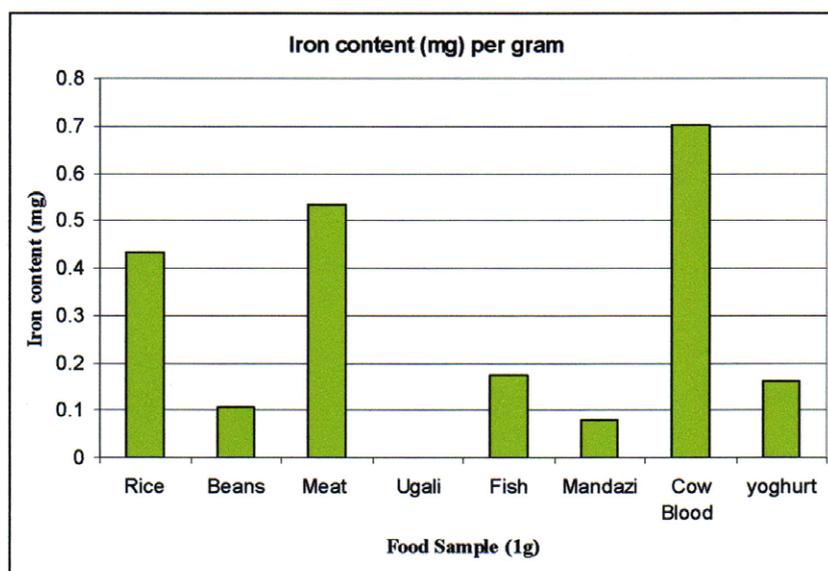
It's evident that the cow blood and meat have the highest Iron content. However cow blood's data may have been altered since it was combusted. Cooking food may have increased the iron content because it's easier to extract from cooked food, but the Masaais have cow blood in its raw state. Rice had high iron content as well, this may have been an anomaly, perhaps this is the true value of Iron content. The lowest iron rich foods are Mandazi and Ugali, Ugali is almost negligible, both these are white flour based food.

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UNCERTAINTIES:

In every experiment, uncertainties and errors exist, this experiment was complex and sources of error come from wide ranges some possibilities could be:
- Uncertainties from materials ex: Weighing Scale
- Preparation of food samples, too much combustion lead to burning up of Iron in the food samples.
- Too little combustion caused some of the Iron to be tightly bonded and hard to break it and thus extract it, reduce Iron content levels.
- Limited sufficient data (narrow range of calorimeter curve)
- Diets taken just for two people in each category, this may not have been a good generalization for all the people in each category especially minimum wage Dar Es Salaam workers come from many different backgrounds and many combinations of diets exist.

I determined the uncertainty using the uncertainties in the values from which the quantity is calculated. Extreme values were used to calculate the extremes in the quantity.

For Calibration Curve:

For concentration of 0.0005 :

Value	Quantity
Transmittance ±0.05	Absorbance
24.66	0.608
24.66 + 0.05 = 24.71	0.609
24.66 - 0.05 = 24.61	0.607

Hence uncertainty in Absorbance= ± 0.01

$$\text{Absorbance} = 210[conc] + 0.600$$
$$(+)\ \text{Absorbance} = 200[conc] + 0.601$$
$$(-)\ \text{Absorbance} = 222[conc] + 0.599$$

These three curves, compensate for any error, some of the points of the initial data aren't exactly on the best fit line, but between the ranges of these lines.
Thus Calibration curve

Absorbance	Concentration $*10^{-5}$
0.608	0.43
0.608+0.01=0.609	0.48
0.608-0.01=0.607	0.38

So uncertainty in concentration= ± 0.5 $*10^{-5}$

Effect on food sample : Error Analysis for rice:

Concentration	Iron Content/ 2.1g $x\ 10^{-4}$
0.00164	0.915
0.00164+0.0000048=0.001645	0.919
0.00164-0.0000048=0.001635	0.913

So Iron content= ± 0.0028mg/2.1g

| Weight	Iron Content/ g $\times 10^{-4}$
2.1	0.4357
2.1+0.05=2.15	0.4274
2.1-0.05=2.05	0.4453

Thus Iron Content/ g of food sample= ± 0.01mg/g

This may seem like a small difference but it does alter the data significantly because Iron content values are small , a small difference changes those small values to be much smaller or much greater.

Using the Iron content per unit of food , I calculated how much Iron each person consumed per day after knowing how much of each food type they consumed. Again I'm assuming this is a typical diet but this isn't everything they eat everyday, but it should give an approximate measure of how much Iron is consumed per day:

Asha	[Mandazi] 25*0.0803= 2.0075 [Beans] 40*0.107= 4.280 [Ugali] 60*0.0000025=0.00015 **6.288 mg of Iron consumed per day**
Mzee Saidi	[Mandazi] 25*0.0803= 2.0075 [Rice] 70*0.435= 30.45 [Fish] 50*0.175= 8.75 **41.2075 mg of Iron consumed per day**

Yohana	[Cow Milk] 0.5*1.218=0.609 [Ugali] 40*0.0000025= 0.0001 [Cow Blood] 250*0.702=175.75 [Meat]100*0.534=53.4 **229.76 mg of Iron consumed per day**
Sentol Mtareh	[Yoghurt] 20*0.162=3.24 [Meat Curry] 50*0.534= 26.7 [Cow Blood] 75*0.702= 52.75 **82.665 mg of Iron consumed per day**

Iron Content in different diets
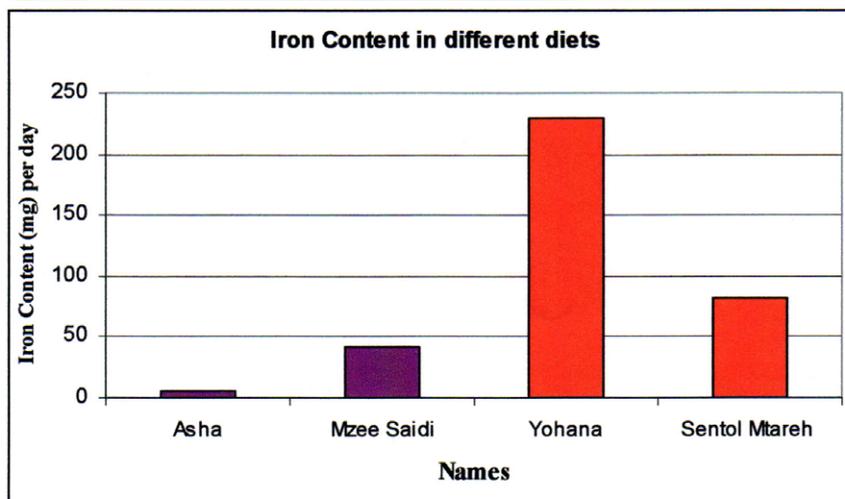
This graphical representation allows us to compare the different diets, distinctively Masaais consume the most Iron. The average ratio is 1:6, where an average amount of Iron consumed is 23.7:156.2 mg, Dar Es Salaam worker: Masaii.

CONCLUSION AND EVALUATION:

How the iron content in diets of different groups of people, the Masaai and minimum wage workers in Dar es Salaam differ, was the aim of this experiment.

Through the use of a calibration, I could compare and find the concentration of Iron in unknown food samples, the case here being Tanzanian local food. The result was for an average minimum wage Tanzanian worker, you would consume around 23.75mg and for a Masaai, the average consumption of Iron per day would be 156.2mg of Iron per day.

Surprisingly both figures are way higher than the RDA figures of 18mg for women and 10 mg for men per day, which contradicts the Iron deficiency statistics in Tanzania. Another observation was that the men consumed much higher amount of Iron per day than the women, however the women were supposed to have a greater Iron rich diet. The lowest amount of Consumption was by Asha, she only consumed 6.288mg per day, which was 35% lower than the RDA value, and the rest all consumed around 785% more than the RDA value. Her diet was dominated by white flour products, from mandazi to ugali, and through experience I would say her diet is most typical of locals in Dar es Salaam including myself. White flour is very low in Iron content as my results showed with only 2.5×10^{-6} mg per gram of ugali.

The main aim was to look at the differences between the two groups, and looking at their average daily intakes, the Masaai have a much greater intake, there's a difference of about 712 mg between the two groups and a ration of 1:6. This show, that cattle based diets help tackle the issue of iron deficiency anemia. Due to their strong Iron diets the Masaai have a strong immune system able to survive in the wild, and have adapted to the harsh surroundings. However because they only feed on cattle based products, the Masaai are limited in other nutrients and may mean that they have other deficiency of other nutrients. On the other hand minimum wage workers seemed to have a greater struggle with income than the masaais have with cultural barriers. This research showed that income is the greatest problem in tackling Iron deficiency anemia, because the whole population is used to feeding on white flour products and less of meat because white flour is cheaper than even whole grain flour, which has a much greater iron content. Perhaps the tradition of diets due to income problems initially has caused an ongoing problem with Iron deficiency anemia. IDA is a major problem in Tanzania, "it is the most widespread nutritional disorder in Tanzania affecting 7.2 million People (32% of the population), 45% of children under five years, and 80% of pregnant and lactating women" [6].

This may have been because of errors or assumptions that could alter the data. Firstly the rice value is wrong, preparing of the rice sample could have gone wrong and thus gives a big value. Rice being an anomaly greatly affected the diet of Mzee Saidi, and gave a big value. Another one is cow blood, even though it should be the highest the value seemed to be too big and thus I think the Masaai data was greatly altered.

Initially I wasn't aware about what the Iron content range would be so I started of with a very narrow range of known concentrations; this affected the equation I derived for the calibration curve. It was the main determinant for the Iron content in food samples. For example when you look at 0.0005 Moldm^{-3} the absorbance is 0.608. When re-applying it in the equation you get a concentration of 0.000043, this is a huge difference when it comes to calculating what the mass of Iron is in the sample. More concentrations would give a greater range of absorbance, thus the equation would be much more accurate.

Preparing the food samples, combustion the hardest part. To make sure whether the food is completely combusted and not less was hard. It caused some iron to be burnt in others, some

very hard to extract from since it wasn't completely combusted. New questions emerged from his research; It would be interesting to see whether cooked food state or raw food state would have a difference in its Iron values. Or whether rice lost some of its Iron when boiled. I started of with the food samples straight away without a trial to look at what problems I may have to face, I ended up not knowing to what extent I had to combust the food, it took a lot of time to make solutions out of the food samples. When filtering out the products I left it for a long enough period of time but perhaps the iron yet hadn't entered the solution.

Spectrophotometer was something new to me, to use it was initially hard but very little inaccuracy could have occurred, because it's very accurate giving me four readings per sample. When collecting food samples, this was based on only two people per group, I think that many more people, more interviews would give me a much better average iron intake per day value, and it would be more representative of each community of people, but also interviews about diets of people through a longer period e.g. a week would be better. This especially affected the Tanzanian minimum wage workers because they come from all different backgrounds and it's hard just to generalize all their diets with just two people. To reduce the risk of loss of iron in the food samples, leaving the Iron to dissolve into the KSCN and HCL for a longer period of time would be beneficial.

Word Count: 3932

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Cover Photos:

Justinsomnia. 5 Oct. 2006 http://justinsomnia.org/gallery/albums/tanzania/DCP_4269.jpg.