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Dietary Iron Determination by UV-Visible Spectrophotometry in Beans, Egg, Fish, and Pork Sold in Makurdi, Nigeria

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Abstract: ***The research aimed to determine the dietary iron levels in pork, fish, egg, and bean samples using the UV-visible spectrophotometric method after acid digestion. Results indicated that beans had the highest dietary iron content (0.750 absorbances), followed by fish (0.304), egg (0.263), and pork (0.256). These findings suggest that all the samples are suitable iron sources and are recommended as supplements for meeting iron requirements. Nevertheless, consuming these iron-rich foods in appropriate quantities is crucial to meet specific dietary needs for maintaining good health. This study's primary objectives were to ascertain and compare the iron content in beans, egg, fish, and pork samples available at the Federal University of Agriculture, Makurdi's student market.***

Keywords: Dietary iron, UV-visible, spectrophotometric, sources of iron

1. Introduction

Iron is a necessary component of hemoglobin and myoglobin, facilitating oxygen delivery, transitional tissue storage, and cellular usage. It also plays critical functions in cytochromes inside mitochondria, facilitating electron transmission in the electron transport chain. Endogenous chemicals and environmental toxins are degraded by Cytochrome P450 in the liver and gut. Iron is also an enzymatic cofactor for aconitase, NADH dehydrogenase, succinate dehydrogenase, and -glycerophosphate dehydrogenase, as well as a component of heme-containing enzymes such as catalase, xanthine oxidase, and glutathione peroxidase. Iron is absorbed in the form of ferrous iron.(Kelly et al., 2023)

Anemia is caused by a low iron content in the body; our bodies need the proper amount of iron. Low iron levels may result from blood loss, a poor diet, or an inability to absorb enough iron from food. Excessive iron consumption has the potential to damage the human body. Excessive usage of iron supplements might result in iron toxicity. Young children and women who are pregnant or menstrual are at a higher risk of having insufficient iron levels. Certain groups, such as young newborns and pregnant or menstruating women, are especially vulnerable to low iron levels. (Camaschella, 2019)

Iron is an essential micronutrient for all living organisms, including humans, and is imperative for the biosynthesis of heme proteins, which serve a vital function in the transportation of oxygen and oxidative metabolism. Iron is ubiquitously found in both plant and animal-based dietary sources, and it may also be detected in the composition of drinking water. There are three distinct forms in which it may be observed: iron oxides, inorganic and organic salts, and organic complexes such as haem iron. Iron fortification has the potential to augment iron consumption; but, in some nations, such as Ethiopia, iron intake may be elevated as a result of food contamination during the preparation process. In the context of Ethiopia, it has been observed that the process of harvesting and threshing cereal grain may lead to the inadvertent contamination of the grain with soil that is rich in iron. This unintended contamination has the potential to significantly increase the daily intake of iron, reaching levels as high as 500 mg per day. The Bantu diet is notable for its substantial iron content, mostly derived from cooking utensils and fermented alcoholic drinks. (Silvestri et al., 2023)

Iron is a crucial mineral that holds significance in our dietary intake. Despite being classified as a trace mineral, necessitating relatively small amounts, the absence of iron in diets can lead to the development of anemia, a deficiency condition. Several natural sources of iron include raisins, liver, and spinach. Additionally, certain food items like breads and cereals are fortified with supplementary iron. (Piskin et al., 2022)

The impact of inadequate or excessive exposure to certain elements on human health is well recognized. The impact of an element is determined by many attributes, including absorption, metabolism, and the extent of engagement with physiological systems. Iron is a crucial element for almost all living creatures due to its involvement in a diverse range of metabolic activities, such as the transportation of oxygen, the creation of deoxyribonucleic acid (DNA), and the facilitation of electron transport. Nevertheless, due to the potential formation of free radicals by iron, it is essential to maintain strict regulation of its concentration inside bodily tissues. This is crucial since high levels of iron may result in detrimental tissue damage. Disorders related to iron metabolism are prevalent in the human population and cover a wide range of illnesses that exhibit various clinical symptoms. These conditions span from anemia to iron excess and may extend to neurodegenerative disorders.(Long et al., 2023)

The examination of the iron (III) ion is conducted in a liquid medium and relies on the subsequent chemical reaction:The aqueous solution of Fe3+ reacts with the aqueous solution of SCN- to form the aqueous solution of Fe(SCN)2+.The intensity of the iron (III) thiocyanate ion's deep red hue is directly proportional to the initial concentration of iron (III) in the solution. The iron content found in various dietary sources exists in the chemical forms of either iron (II) or iron (III). (Waddell et al., 2023)

$$Fe^{3+}(aq) + SCN^{-}(aq) \rightarrow Fe(SCN)^{2+}(aq)$$

Iron, when ingested in appropriate quantities within the human diet, offers a multitude of advantages, which will be further upon in the subsequent sections. (Charlebois & Pantopoulos, 2023).

(i) Boosts hemoglobin formation (ii) Improves muscle function (iii) Increases brain function (iv) Treats restless leg syndrome (v) Regulates body temperature (vi) Oxygen carrier (vii) Treats anemia

Iron deficiency is a prevailing nutritional insufficiency on a Worldwide scale. The prevalence of the condition is highest among individuals in the early stages of childhood, expectant mothers, and women within the reproductive age range. The first stage of iron shortage is characterized by the gradual depletion of ferritin, which represents the body's tissue iron reserves. At this stage, the manifestation of clinical symptoms is quite low. As the severity of the iron shortage escalates, there may be observable functional repercussions, including compromised immunological function and diminished workability. Severe iron deficiency is characterized by the presence of microcytic hyperchromic anemia, resulting in symptoms such as listlessness, weariness, and breathing, palpitation during physical activity, and diminished workability. Iron deficiency anemia has been linked to compromised behavior and cognitive development throughout infancy and early childhood, as well as reduced susceptibility to infections. Additionally, altered temperature control has been seen in individuals with iron deficiency anemia. (Kumar et al., 2022)

The physiological requirements of the person and the bioavailability of the dietary iron consumed both have an impact on the determination of recommendation intakes. Age, sex, and food type are used to categorize the recommended intakes. The dietary recommendations for women in the reproductive age range are high in order to properly meet the nutritional needs of people who have significant menstrual blood loss. At the same time, it is clear that certain women might not need to consume as much. Even though iron is easily accessible, the nutritional needs for iron during the second and third trimesters of pregnancy cannot be efficiently met only by food sources. Unless there is an initial storage of around 500 mg of iron at the beginning of pregnancy, iron supplementation is required.(Billaut et al., n.d.)

2. Materials and Method

Reagents
Ferric ammonium sulphate $FeNH_4(SO4)_2$ 1.205g, ammonium thiocyanate (NH_4SCN) 38.0 g, sulphuric acid (H_2SO_4) 55 mL, Hydrochloric acid 85.0 mL, Distilled water 5 Litres. (K.C Industrial Chemicals North Bank Makurdi)

Apparatus
UV-Vis spectrophotometer Cole-ParmerJenway Model 7415, 250 mL beaker (Griffin beakers), 250mL volumetric flask (Type A), 1 L volumetric flask (Type A), 100 mL Conical Flask (EISCO), 10 mL Measuring Cylinder (EISCO), mortar and Pistils, Crucible(LabZhang), Test Tubes (Fubchem), Hand Gloves and safety glasses (Dre Health)

Preparation of solutions

Preparation of 0.001 M ferric ammonium sulphate ($FeNH_4(SO4)_2$)
Exactly 1.205g ferric ammonium sulphate was weighed, grinded in a mortar and dissolved in 10mL concentrated sulphuric acid (S.G 1.84; 98%) in a beaker. The solution was left over night for proper dissolution. Thereafter, it was poured into a 250mL beaker and made to mark with distilled water. Then 20 mL of the solution was transferred into another 250 mL volumetric flask and made to mark with distilled water to give a standard solution of 0.001M. (Tyner & Francis, 2017)

Preparation of standard solution for calibration curve
To prepare a 4 $x10^{-5}$M standard solution, 10.0 mL of 0.001M $FeNH_4(SO4)_2$ was measured into a 250 mL volumetric flask and 10.0mL of 1.0M sulphuric acid was added. The solution was made up to mark with distilled water. This same procedure was repeated by measuring 20, 30, 40 and 50mL of 0.001M $FeNH_4(SO4)_2$ solution in separate 250 mL volumetric flasks to obtained 8 x 10^{-5},1.2 x 10^{-4},1.6 x 10^{-4} and 2.0 x 10^{-4} M standard solutions respectively.

Preparation of 1.0M Ammonium thiocyanate solution
Exactly 38.0g of ammonium thiocyanate was weighed and grounded into a beaker then, 100 mL distilled water was added, the solution was made to dissolved and later transferred into a 500mL volumetric flask and made to mark with distilled water.

Preparation of 1.0M sulphuric acid
Exactly 55 mL of sulphuric acid (S.G 1.84; 98%) was measured and transferred into a 1 L volumetric flask. The solution was made to mark with distilled water.

Preparation of 1.0M HCl solution
Exactly 85.0mL of Hydrochloric acid (S.G 1.18; 36.5%) was measured into a 1 L volumetric flask and made up to the mark with distilled water.

Method of Sample Collection
The flesh freshly sacrificed pork and fish, were purchased from the student's market at the Federal University of Agriculture Makurdi. Beans and eggs were also purchased. Samples were packaged in polyethylene bag and transported to the laboratory after thorough washing with water. Samples were stored in a freezer at the temperature of 60^{o}c prior to analysis(*Meats/Meat Alternates Food Buying Guide for Child Nutrition Programs*, n.d.)

Preparation of samples
Exactly 0.30g each of the samples (fish, egg, pork meat and beans) was taken weighed into a 50 mL crucible and covered. The samples were properly labeled and transferred into a muffle furnace. These were ashed at 300 ^{0}C for three hours to obtain the ash.

On cooling, the samples were transferred into a beaker and 10.0 mL of 1.0M HCl was added and stirred for 5 minutes

using a magnetic stirrer. Thereafter, 5.0mL of distilled water was added and the solution was filtered into a 50 mL volumetric flask and diluted to the volume with distilled water. Sample concentration = 0.3g/50 ml = 6g/L = 6000mg/L.

Preparation of calibration curve

Exactly 10mL of each Fe^{3+} standard solution was measured into separate labeled test-tubes in order of increasing concentration then 10 mL of ammonium thiocyanate was added in each test-tube containing iron. The solution was mixed by swirling and allowed to stand for 10 minutes with a stable change in color form colorless to red. After 10 minutes the absorbance was measured at 490nm. The absorbance obtained from the standard solutions was used in plotting a calibration curve.

Now the question arises, how can the concentration of an unknown sample be determined just by measuring the amount of light it absorbs? Here the "law of absorption" plays a crucial role and clears the doubts.

Beer-lambert law (or beer's law) states that there is a linear relationship between the absorbance and concentration of a sample. For this reason, beer's law can only be applied when there is linear relationship. The equation is;

$A = \varepsilon bc \ldots\ldots\ldots\ldots\ldots equation\ (i)$

Where ;A =absorbance (no unit)

ε =molar absorptivity

b = path length of the sample(i.e the path length of the cuvette in which the sample is contained (cm)

c = the concentration of the analyte in the solution (mol/L)

Experimental measurements are usually made in terms of transmittance, T, which is

$T = I/Io \ldots\ldots\ldots\ldots\ldots equation\ (ii)$

Where; I =intensity of light after it passes through the sample, and

Io = initial intensity of light.

The relationship between Absorbance, A and Transmittance, T is

$A = -log\ T\ = -log\ (I/Io) \ldots\ldots\ldots\ldots\ldots equation\ (iii)$

The spectrophotometer displays either %transmittance or absorbance. Thus unknown concentrations of analyte (sample) can be determined by measuring the amount of light the sample absorbs by applying Beer's law. If the absorptivity coefficient is not known, then the unknown concentration can be determined by using a working curve of absorbance versus the concentration derived from the standards (Namrata ,. 2013).

Analysis of sample

In the same vein, 10mL of each sample was measured into labeled test-tubes, followed by the addition of 10 mL of 1.0M ammonium thiocyanate for colour development. After 10 minutes, the absorbance was measured at 490 nm. The concentration of iron in the food sample was extrapolated estimated using linear curve obtained from the calibration curve.

3. Result and Discussion

3.1 Results

The analysis revealed that every single one of the chosen protein-rich food samples included a significant level of iron. The amount of iron contained in various food samples was variable, with beans having the greatest iron content, followed by pork, fish, and eggs in descending order of iron concentration.

Table 1: Concentration of Iron in (mg/kg) in Various Samples

Sample wt. (g)	Sample name	Mean absorbance	Fe (g/g)	Fe (mg/kg)
0.31	Fish	0.304	5.8506E-5	0.00377
0.31	Egg	0.263	5.0615E-5	0.00326
0.31	Pork	0.256	4.9461E-5	0.00317
0.30	Beans	0.750	1.4434E-4	0.00962

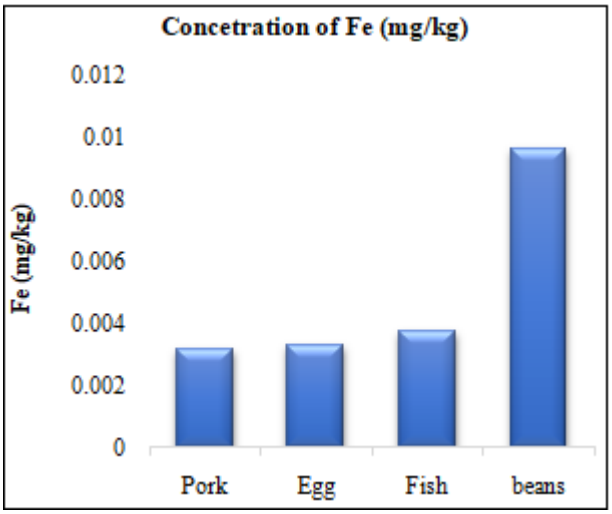

Figure 2: Concentration of Fe (mg/kg) in selected Food Items

4. Discussions

The study results demonstrate that the selected protein-rich food items, such as beans, pork, eggs, and fish, have substantial iron content. The stability of the red color complex generated persisted for many minutes, during which absorbance measurements were obtained using a UV-visible spectrophotometer (Jenway Model 7415) set to a wavelength maximum of 490 nm. The wavelength of a UV-visible spectrophotometer is typically determined by the absorption characteristics of the chemical or chromophore. A compound exhibiting a red hue is seen, with the highest level of light absorption occurring at a wavelength of 490 nm. In the field of UV-visible spectroscopy, this technique is used to accurately measure the absorbance of a given analyte at its most prominent wavelength. The findings indicate that there is a positive correlation between absorbance values and concentration, aligning with the principles outlined in the Beer-Lambert equation.

The analysis of the iron experiment used Beans, Egg, Fish, and Pork as the primary subjects for investigation. The concentration values of the different food samples are as

follows: beans (0.00962), fish (0.00377), egg (0.00326), and pork (0.00317), with beans having the greatest concentration and pork having the lowest concentration. The sample of beans exhibits the greatest absorbance and the highest quantity of iron among the aforementioned samples. This observation may be ascribed to the specific environmental conditions and soil composition.

The concentration of iron in beans from this study was0.00962. Iron concentration as reported by (Golam *et al.*, 2011). has the value of 8.9mg/kg for beans. The result obtained when compared, shows a very high iron concentration.

Fish has an absorbance value of 0.304 and the concentration of iron from this study is 0.00377 mg/kg. The result obtained from this work is line with the study of (Jessica *et al*., 2015), which report iron concentration of fish in Bangladesh as 0.34mg/100g (340 mg/kg). The above result when compared to the 11.9-20mg/100g range for iron in fish (WHO,. 2017) shows a greater range difference in content. The difference may be due to the nature of water or environmental changes.

Egg has the absorbance value of 0.263 and iron concentration of 0.00326 mg/kg from this study. A similar study by (Vilija *et al*,.2016) reviewed 150 mg/kg iron content in eggs. (Cornescu*et al*,. 2014) also found out 120 mg/kg concentration of iron in Egg. The difference between the works of Vilija*et al*(2016) and Cornescu*et al*,.(2014) and my research work is due to the iron feed supplements given to the Hen used for the research work of Vilija*et al*,.(2016) and Cornescu*et al*(2014).

Pork has the absorbance value of 0.256 and an iron concentration of 0.00317 mg/kg from this work. The work of Greenfield *et al* (2009). On Australian pork report 4.60 mg/kg concentration of iron in the pork. The work of Greenfield *et al*,.(2009) has higher concentration of iron in pork. This result may be due to the iron knives used in the slicing of the pig.

5. Conclusion

The dietary iron in pork, fish, beans and egg samples were determined using UV-Vis Spectrophotometric method of analysis. The result from the study revealed that beans sample has the highest value of dietary iron 0.00962 (mg/kg) followed by fish 0.00377(mg/kg) followed by Egg 0.00326(mg/kg) and the least value was estimated for pork 0.00317(mg/kg). from this research work, it was concluded that all samples are good sources of iron supplement for body. and also advisable for pregnant women, infants, adolescent and adult, especially those that undergo menstruation should acquire more iron from selected food sample such as those considered in this research.

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