A PHYSICOCHEMICAL EVALUATION ON THE EFFECTS OF PARACETAMOL IN COOKING COW BEEF (Bos Taurus)

BY

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JANUARY, 2023

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A DISSERTATION TO THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARMD DEGREE)

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY

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CERTIFICATION

This is to certify that this project work was carried out by **Deborah Osamudiamen OGEDENGBE**, in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

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Dr. E.E. ODION DATE
(HEAD OF DEPARTMENT)

DEDICATION

This research is dedicated to God Almighty, who has been my source of inspiration throughout my sojourn through Pharmacy school and to my dear parents, Elder and Deaconess Francis Ogedengbe for their unwavering support.

ACKNOWLEDGEMENT

I thank God almighty for his continuous guidance, wisdom and sufficient grace. Special thanks to my Parents, Elder and Deaconess Francis Ogedengbe for their steady support and to my amazing partner, Barr. Eric Osemwengie for his unwavering support. I thank my highly esteemed supervisor, Prof Patrick Igbinaduwa for his mentorship and guidance. I will not also fail to acknowledge every staff and student of the Faculty of Pharmacy, University of Benin, who was of help to me in one way or another throughout my journey in Pharmacy school. Finally, I thank my siblings, family members and friends for being a pillar of support, God bless you all.

TABLE OF CONTENT

ABSTRACT

Paracetamol also known as Acetaminophen or N-acetyl-Para aminophenol (APAP) is one of the most widely used non-prescription medications known for its analgesic and antipyretic property. The experimental procedure involve standard methods of analysis and was focused on the determination and comparison of specific nutritional or related parameter. The parameters include; pH, moisture, protein, fat, ash and essential micro-nutrients (Calcium, potassium, sodium, Iron, zinc, magnesium) when cow beef was cooked with paracetamol and without paracetamol. The PH3C pH meter was used to determine the cooked solution pH, Protein content was determined using the Kjeldahl method of protein determination, The fat content was determined by Soxhlet extraction with n-hexane, the loss on drying method was utilized to determine the moisture content, a heating furnace was used to determine the amount of ash in the sample and the ash was further analyzed for micronutrients with the aid of atomic absorption spectroscopy (Buck scientific 210VGIP Atomic absorption spectrometer).

The result from the study showed an increase in the concentration of moisture, fat, ash and micronutrients in beef cooked with Paracetamol and a decrease in the protein content and pH values of beef cooked with Paracetamol. This study demonstrated that beef cooked with Paracetamol and less protein content but higher levels of fat and metal ions.

CHAPTER ONE

1.0.INTRODUCTION AND LITERATURE REVIEW

1.1.Introduction

Paracetamol also known as Acetaminophen or N-acetyl-Para aminophenol (APAP) is one of the most widely used non-prescription medications in the world indicated for the treatment of pain and fever. It is most readily available and affordable. (Ahmed, 2020)

A decline in the use of Aspirin in the 1980's as a result of its connection with Reye's syndrome allowed for the use of Paracetamol as the major antipyretic and analgesic drug of choice in children and presently, Paracetamol has become the standard antipyretic and analgesic drug used for all ages (Graham, 2013). Paracetamol is better tolerated than the non-steroidal anti-inflammatory drugs (NSAIDs), although it may be less efficacious because of its unclear mechanism of action and non-selectivity to the Cyclooxygenase 2 Pathway (COX-2). Paracetamol, although being a safe and efficacious drug has inconveniently high doses with a maximum daily dose of 4g which allows for more tablets to be taken and as such, increasing the potential for over dose and drug toxicity.

Paracetamol as earlier stated is safe but less efficacious when compared with NSAIDs, the table in **fig 1** below further shows the pharmacological and clinical activities of paracetamol in comparison with NSAIDs.

Pharmacological activity	Paracetamol	Selective COX-2 inhibitor	Non-selective NSAID
Analgesia	Active	Active	Active
Antipyresis	Active	Active	Active
Anti-inflammatory	Active in mild inflammation	Active	Active
Anti-platelet	Low activity	Inactive	Active
Damage to stomach and small intestine	Low activity	Low activity	Active
Aspirin-induced asthma	Weakly active	Inactive	Active
Blood pressure	Variable data	Increase	Increase
Renal	Lesser effects than both NSAID classes	Impaired function in stressed kidneys	Impaired function in stressed kidneys
Increased risk of thrombosis	Inactive	Active	Active

Fig 1; Summary of pharmacological and clinical activities of paracetamol, selective COX-2 inhibitors and non-selective NSAIDs

Most food contains a mixture of some or all of the available classes of nutrient. Some of these nutrients can be stored internally (Fat soluble vitamin) while the others are required more or less continuously.

Micronutrients (vitamins and minerals) have numerous health benefits including tissue maintenance, bone and teeth formation and health, serving as cofactors and coenzymes to enzyme various enzyme systems, aiding the regulation of most body functions, as well as other physiological and biological functions in the body. Micronutrients are essentially required by human and other organisms in varying amounts throughout life to coordinate various physiological functions to maintain health (Gernand, 2016)). Humans as well as other animals require several vitamins and minerals for daily function (Blancquaert, 2017). Micronutrient requirements in humans are usually in amounts below 100 mg/day, in contrast to macronutrients which are required in grams per day. The minerals for human and animals include 13 elements

such as calcium and iron which originate from the soil and cannot be synthesized by living organisms (Corvallis, 2018). Human micronutrient requirements also include vitamins, which are organic compounds that are usually required in micrograms or milligrams. Since plants are the main origin of nutrients for humans and other animals, some micronutrients can be in low quantities and deficiencies can occur when dietary intake is insufficient as occurs in malnutrition, indicating the need for necessary measures to prevent inadequate supply of micronutrient in plant foods as well as including the combinations of foods and flours to ensure nutrient complementation, this commonly practiced in food industries. (Corvallis, 2018)

It is essential to quantify the dietary nutrients that can be obtained from common foods and to ensure that the foods meet specific nutritional or related requirements that are suitable for human consumption

1.2. LITERATURE REVIEW

Paracetamol is widely known for its analgesic and antipyretic properties (Ahmad, 2018). It is used to relieve mild to moderate pain, which include headaches, sprains or toothache and alleviate fever caused by certain illnesses like flu and colds

Paracetamol is often recommended as one of the first treatments for pain, as it safe for most people with more or less rare side effect. (Graham, 2013)

Chemically, Paracetamol is a phenol and is readily oxidized like other phenols to Quinones as seen below in **fig.4**. This oxidation is key to the postulated mechanism of action of Paracetamol as a substrate and an inhibitor of the peroxidase function of the COX-1 and COX-2 pathway. Paracetamol has a small molecular mass as seen in **fig.2** below; it is a weak acid with a pKa of 9.7 and is unionized at Physiological pH values.

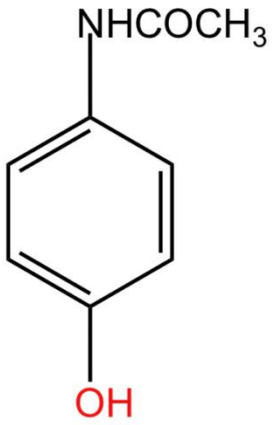

Fig.2; chemical structure of Paracetamol (acetaminophen)

1.2.1. Paracetamol Synthesis

In Paracetamol synthesis, Phenol is first nitrated to Para-nitrotoluene, The o-isomer and the p-nitro group is removed by a steam distillation to form p-aminophenol which is then acetylated to Paracetamol

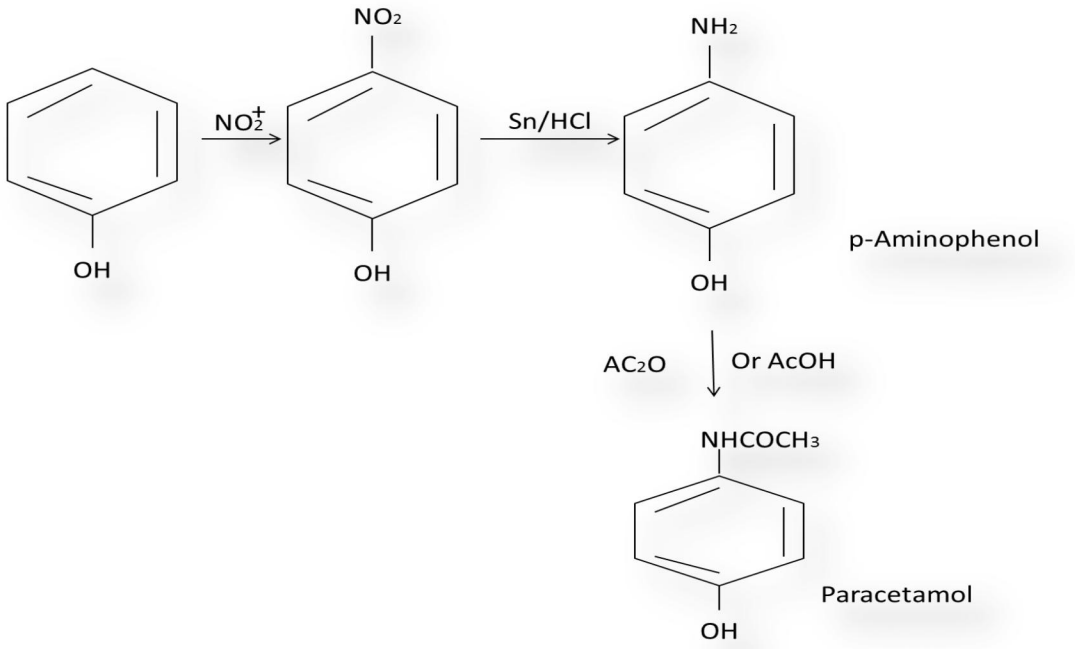

Fig 3; structural elucidation of paracetamol (acetaminophen) synthesis

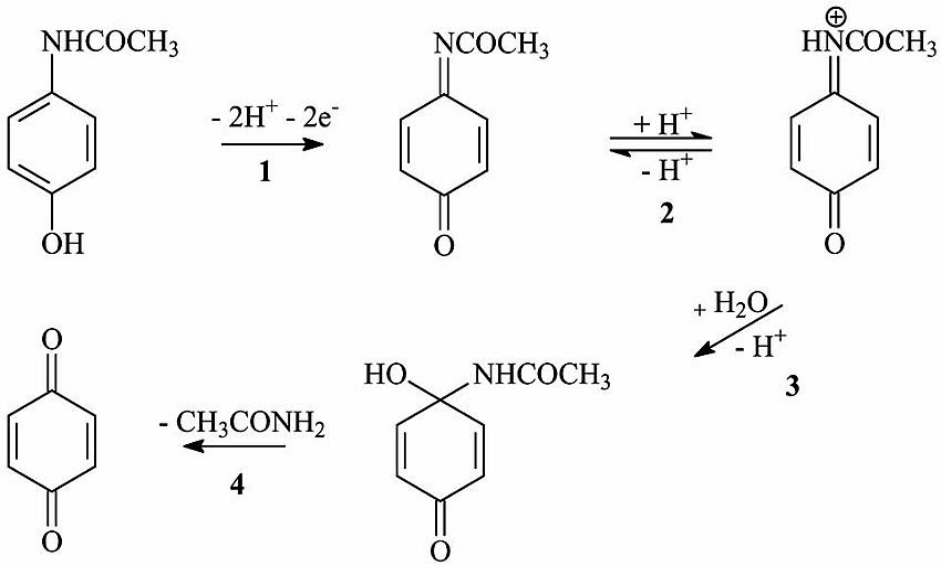

Fig 4; structural elucidation of paracetamol (acetaminophen) oxidation to Benzoquinone

1.2.2. Paracetamol mechanism of action

Paracetamol is known to exhibit a **central analgesic effect** which is mediated through the activation of descending serotonergic pathways (Anderson, 2008)

In the arachidonic acid pathways, paracetamol acts as a **reducing substrate** in the reaction required to convert arachidonic acid to PGH2 on the peroxidase site. Arachidonic acid is first converted to PGG2 after gaining two molecules of oxygen via the cyclooxygenase site, after which PGG2 is reduced to PGH2 via the peroxidase site. This conversion of PGG2 is dependent on the Tyrosine-385 radical at the cyclooxygenase site. The generation of this radical is dependent on the generation of a ferryl protoporphyrin IX radical cation at the peroxidase site. Paracetamol acts as a co substrate which partially reduces the ferryl protoporphyrin IX radical cation, hence, ensuring that less of the cation is available to be transferred to the cyclooxygenase

site, and subsequently, less Tyr-385 is available to sufficiently convert arachidonic acid to PGG2. (Anderson, 2008)

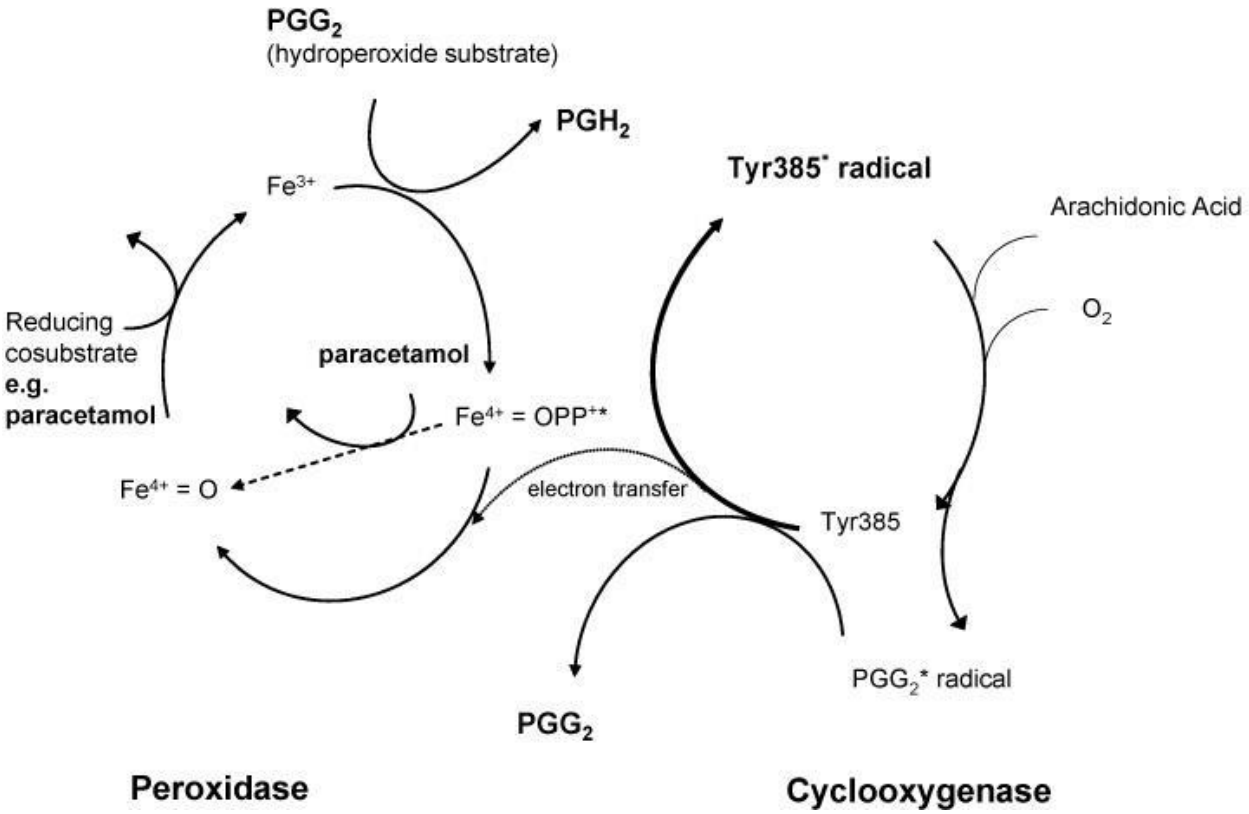

Fig 5; structural elucidation of paracetamol (acetaminophen) mechanism as a reducing substrate

1.2.3. Paracetamol Metabolism

Following oral administration, Paracetamol is absorbed rapidly from the gastrointestinal tract, with a dose dependent systemic bioavailability ranging from 70 to 90%. The rate of oral absorption is predominantly dependent on the rate of gastric emptying, which can be delayed by food, pethidine, diamorphine, propantheline but enhanced by metoclopramide. It distributes evenlyand rapidly throughout most tissues and body fluids and has a volume of distribution of approximately 0.9L/kg. 10 to 20% of the drug is bound to red blood cells. (Forrest, 1982)

Paracetamol is extensively metabolized prevalently in the liver, the major metabolites are the sulphate and glucuronide conjugates. A minor fraction of the drug is converted to a highly reactive metabolite which is readily inactivated in the presence of increased glutathione and excreted in the urine as cysteine and mercapturic acid conjugates. In therapeutic doses paracetamol is a safe analgesic, but when taken in over dose (>4g daily dose), can lead to hepatic necrosis as a result of the depletion of glutathione and the binding of excess of the reactive metabolites to vital cell constituents. This damage can be prevented by the early administration of sulfhydryl compounds such as methionine and N-acetyl cysteine. (Marta, 2013)

In healthy subjects, 85 to 95% of a therapeutic dose is excreted in the urine within 24 hours, 4% appearing unchanged with its glucuronide, sulphate, mercapturic acid and cysteine conjugates, respectively. The plasma half-life in such healthy subjects ranges from 1.9 to 2.5 hours, wereas, the total body clearance ranges from 4.5 to 5.5 ml/kg/min. Glucuronidation is the main pathway of acetaminophen metabolism, followed by sulfation and the oxidation (**fig.4**). Oxidation by cytochrome-P (CYP) iso-enzymes yields a reactive metabolite NAPQI that is detoxified by the glutathione pathway (binding to glutathione to yield readily execrable cysteine and mercapturic

metabolites). Phenytoin and Phenobarbital and inhibit acetaminophen glucuronidation, while ethanol and isoniazid enhances acetaminophen oxidation (Mazaleuskaya, 2015). Therapeutic doses of Paracetamol is metabolized through different mechanisms as seen in **fig.6**

Cytochrome P450 enzymes catalyze the oxidation of acetaminophen to its reactive metabolite N-acetyl benzoquinone imine (NAPQI). The exact contribution of particular CYP isoforms to the bioactivation of Acetaminophen (APAP) varies and depends on the concentration of the drug. In human liver microsomes, CYP2E1 and CYP1A2 were first reported to be responsible for converting high doses of APAP to NAPQI. Later studies, combined purified human liver microsomes with specific inhibitors to confirm the role of CYP2E1 in the bioactivation of toxic levels of APAP, but also report involvement of CYP2A6 (Manyike, 2000)]. Studies with healthy human volunteers pre-treated with the CYP2E1 inhibitor, disulfiram, further confirmed the role of CYP2E1 in APAP oxidation. (Manyike, 2000)

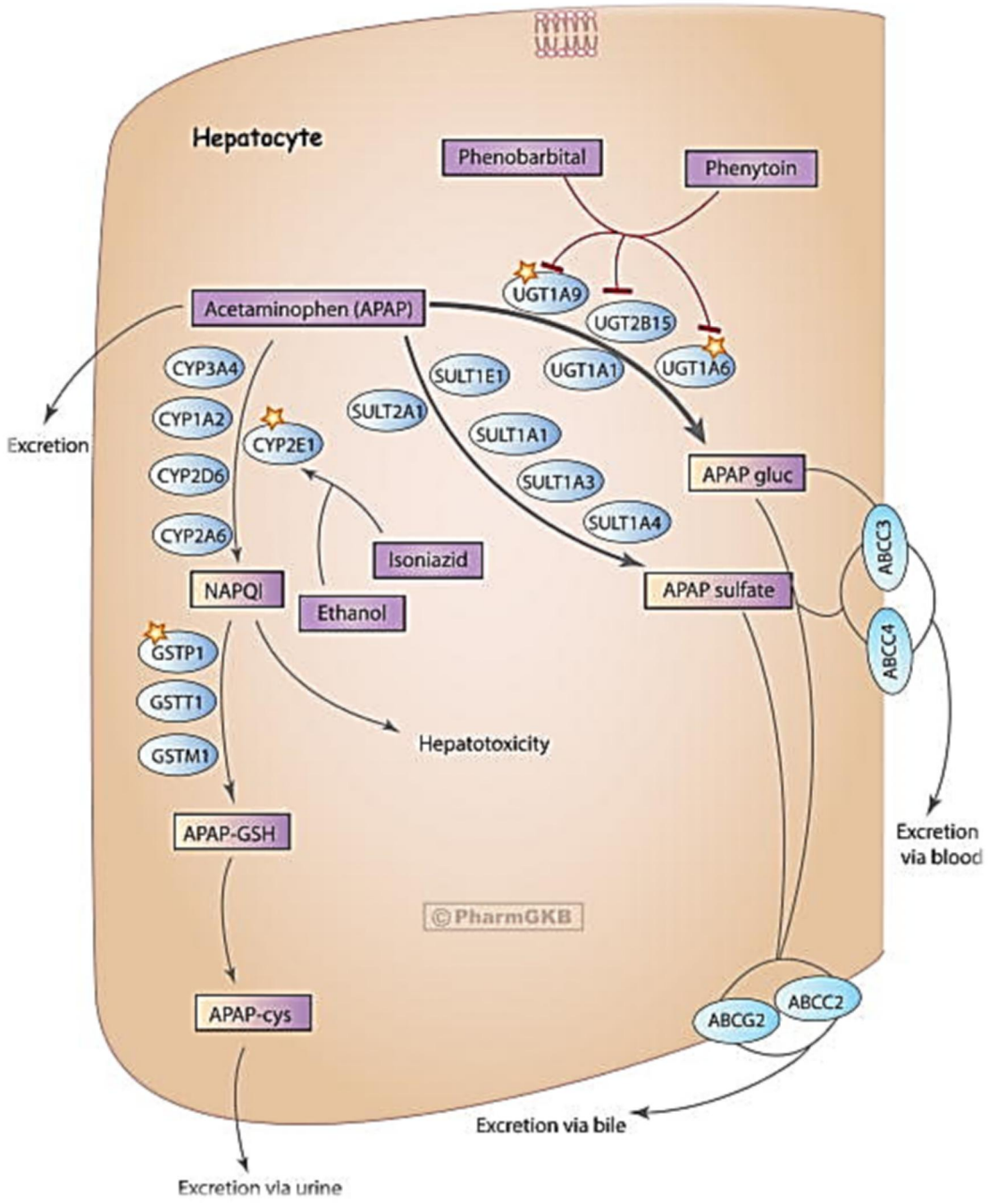

Fig 6; Pathway for acetaminophen (Paracetamol) metabolism at therapeutic doses

1.2.4. Paracetamol Toxicity

The liver, the kidneys and the intestine, are the major organs implicated in acetaminophen metabolism. (Mazaleuskaya, 2015). After a therapeutic dose, APAP is mostly converted to pharmacologically inactive glucuronide (APAP-gluc, 52–57% of urinary metabolites) and sulfate (APAP sulfate, 30–44%) conjugates, with a minor fraction being oxidized to a reactive metabolite N-Acetyl-p-benzoquinone imine (NAPQI 5–10%) fig.4. Less than 5% of APAP is excreted unchanged. NAPQI is a highly reactive metabolite known to be primarily responsible for acetaminophen induced hepatotoxicity. After ingestion of highly toxic doses of acetaminophen, glucuronidation and sulfation pathways get saturated and higher portion of the drug gets oxidized and excreted unchanged. Excess NAPQI depletes glutathione store, leading to liver injury. Administration of NAC provides an exogenous source of glutathione that will neutralize NAPQI and prevent further hepatotoxicity. Enzymes playing a major role in the corresponding pathway are denoted with a star as seen in **fig. 7** below. APAP (acetaminophen), APAP-cys,(acetaminophen cysteine), APAP gluc (acetaminophen glucuronide), NAC(N-acetylcysteine), APAP sulfate, NAPQI(*N*-acetyl-*p*-benzoquinone imine)

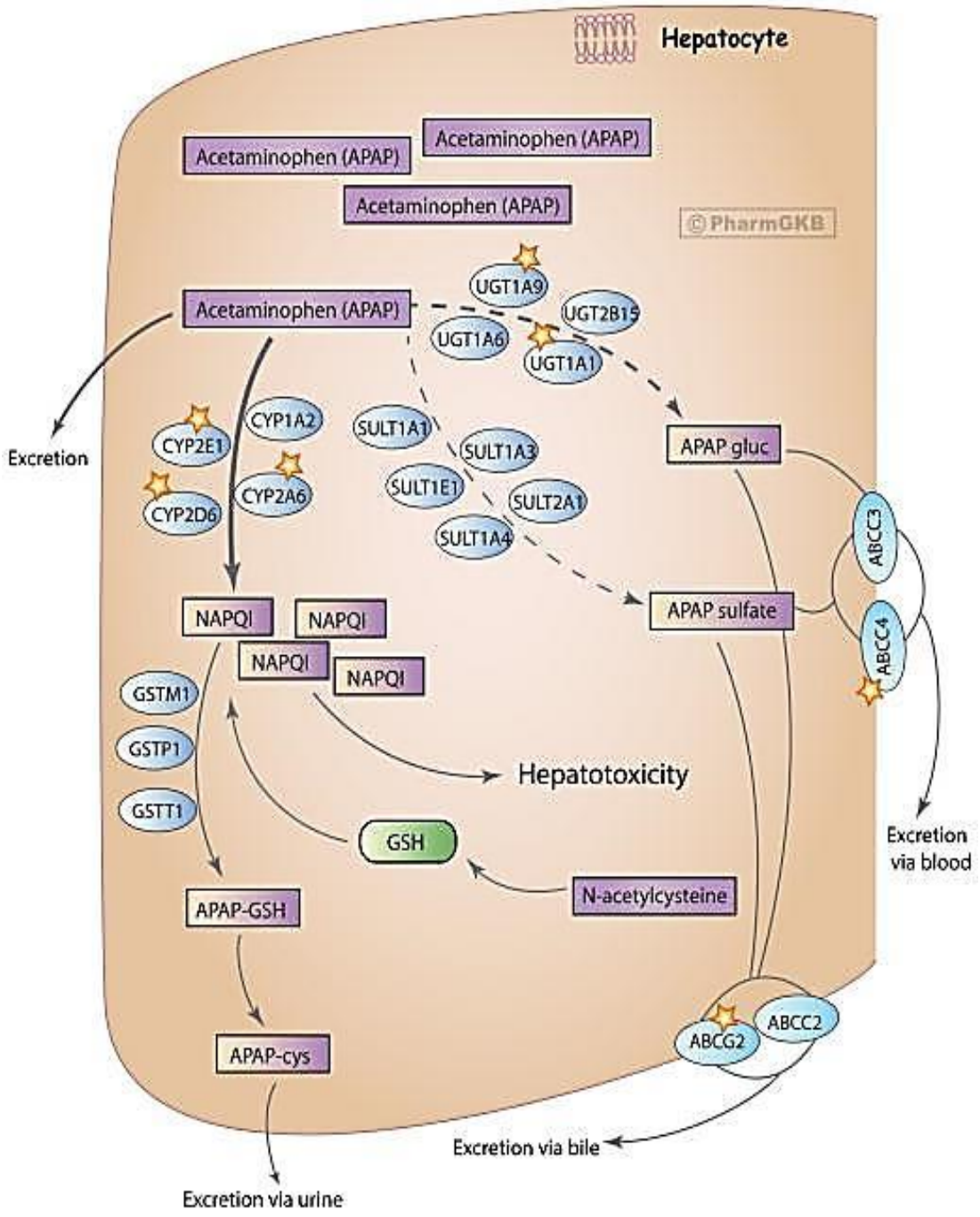

Fig 7; Pathway for acetaminophen (paracetamol) hepatotoxicity

1.2.5. Paracetamol as a meat tenderizer

This topic stems from the discovery that Paracetamol is considered the fastest way to prepare meat in some households and restaurants. It is said to minimize the cost of for procuring cooking resources such as gas, kerosene or firewood and costs much less, hence, it is readily available and affordable for use, as N50 for one sachet of 12 tablets, and each sachet can cause a pot-full of meat to soften within few minutes a young mother, Adeola told this writer upon inquiry. “I have used it to cook on several occasions, it is a lot easier.

In the year 2020, an article was released by the National Agency for Food and Drugs Administration and Control (NAFDAC) warning food vendors and others against the use of Paracetamol as a tenderizer in cooking meat, stating that it can cause kidney and liver failure.

During a sensitization programme for fast food operators and vendors on the dangers of using Paracetamol to cook, Mrs. Roseline Ajayi, the Kwara Coordinator of NAFDAC, stated that “the use of paracetamol to tenderize meat may be considered as a cost friendly method but with grievous and unwanted health implications, of which may include kidney and liver failure. The NAFDAC coordinator, Mrs. Roseline Ajayi, explained that food aims to provide nutritional support and promote life, while a drug is a substance utilized in the cure, treatment, and prevention of certain diseases. She warned that when Paracetamol is used for cooking, it is hydrolyzed and broken down into several degradation components, one of which is highly toxic to the kidney and liver, This degradation component is referred to as **4-aminophenol** (The guardian, 2020)

1.2.6. Nutritional composition of Meat and their Quantification

2. Water

Meat ranks amongst perishable food materials, as it is known to contain around more than 70% of moisture in it and this moisture content determines the shelf life the meat and meat product. (Ahmad, 2018). During meat processing, the water retained within the muscle fiber is fortified with the pressure and temperature and is referred to as “bound water” while most of the water “lost” during these circumstances is known as “free water” (Ahmad, 2018). The water holding capacity of meat can be compromised by disrupting the muscle fibers; this will in turn enhance the shelf life of the meat and related products.

Water is the simplest of all other food constituents and it poses great concerns to manufacturers and consumers. The accurate determination of moisture poses many challenges as it is a difficult task to separate water from food samples and this difficulty can result in underestimation of moisture content. Whereas, utilizing harsh methods to remove all moisture from food samples may cause decomposition of the product and loss in sample mass. Most of the methods used to estimate of moisture content depend on the loss in weight on heating. Analytical methods of moisture determination can be classified in two, of which are the direct methods e.g., oven drying, freeze drying, vacuum drying, desiccation, gas chromatography, thermo-gravimetric analysis and the Indirect methods e.g., NMR spectroscopy, refractometry, infrared absorption, microwave absorption, mass spectrometry and dielectric capacitance.(Greenfield, 2003)

3. Fat

Fat is an important nutrient responsible for providing energy, aiding growth and development, and enabling the body's absorption of fat-soluble vitamins. There are three different types of naturally occurring fats in foods, they include; unsaturated, saturated, and trans fats.

Unsaturated fats are "healthy" fats that are essential components of a well-balanced diet. More than half (55%) of the fat in beef is unsaturated. Most of the unsaturated fat in beef is oleic acid, as seen in olive oil. Beef, like all other animal-based foods such as dairy and poultry, contains saturated fat. Some plant-based foods, like palm and coconut oil, contain large amounts of saturated fats. Approximately 40% of the fat in beef is saturated, of which 13% is stearic acid. Stearic acid does not contribute to low-density lipoprotein (LDL) cholesterol, the "unhealthy" cholesterol.(Moloney, 2002)

About 3% of the fat found in beef is natural trans-fat. Trans fat can also be found in other animal-based foods, however the primary source of trans fat in commercially baked goods and vegetables. Industrial trans fats is unhealthy

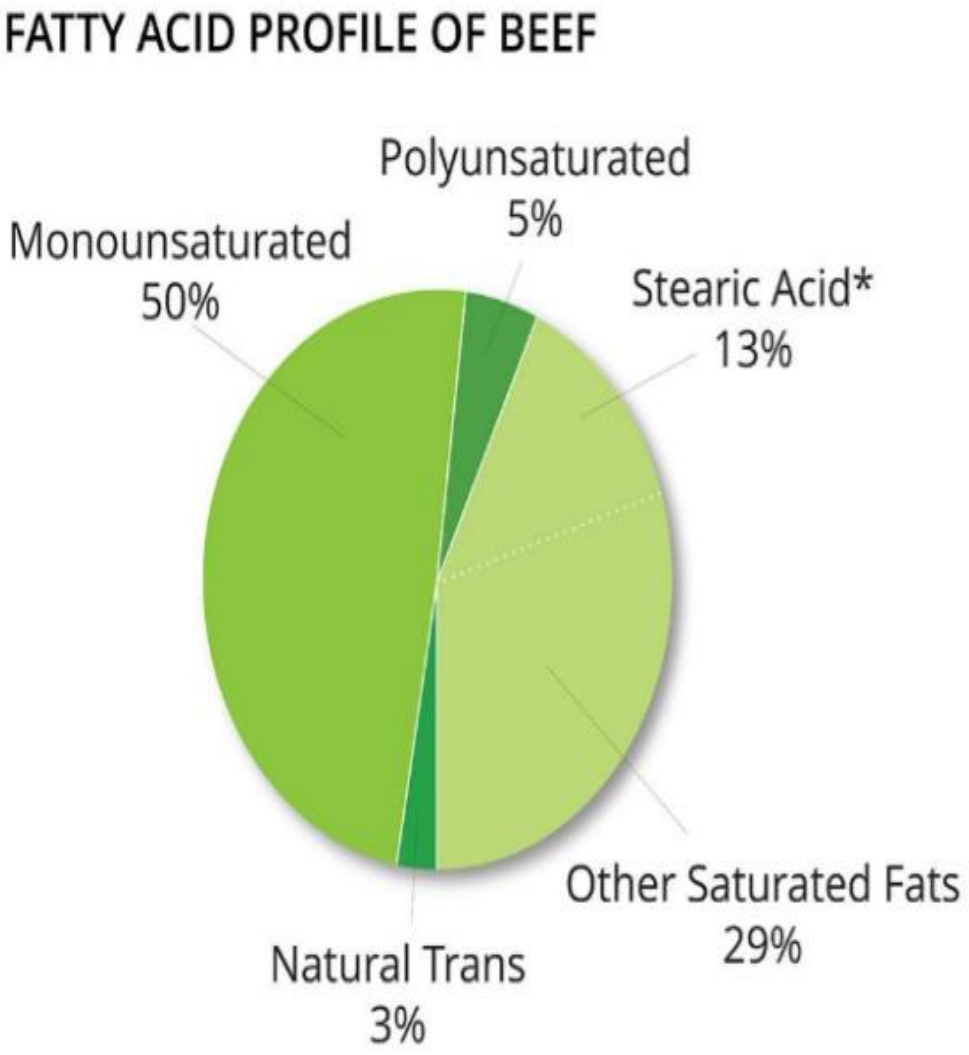

Fig.8; chart representing the fatty acid profile of beef

Flavor is unarguably the most important factor in food choices (Pearson, 1999). “Fat is the source of flavor in meat and is particularly important to the characteristics species flavor variations. Fat serves as a precursor of flavor when in combination with amino acids from proteins and other constituents upon heating. (Pearson, 1999). Lipid content of muscle ranges from 1.5% to 13% (Dinh, 2006). Although most of lipid is present in adipose tissues, lipids found intracellular in muscle fibers have the most important roles in meat quality. Intracellular lipid deposits are called marbling. Marbling refers to intramuscular fat that is deposited within the muscle in connective tissues between muscle bundles (Dinh, 2006). Free fat can be extracted by the less polar solvents such as petroleum ether and diethyl ether, whereas the bound fat requires more polar solvents like alcohols for their extraction. The bound fat may be broken down by hydrolysis or other chemical reaction to yield free fat. Hence, the amount of extracted fat found in food product is highly dependent on the method of analysis used. Soxhlet extraction has been known to show high yield compared to other methods of lipid content determination. (Perez, 2008).

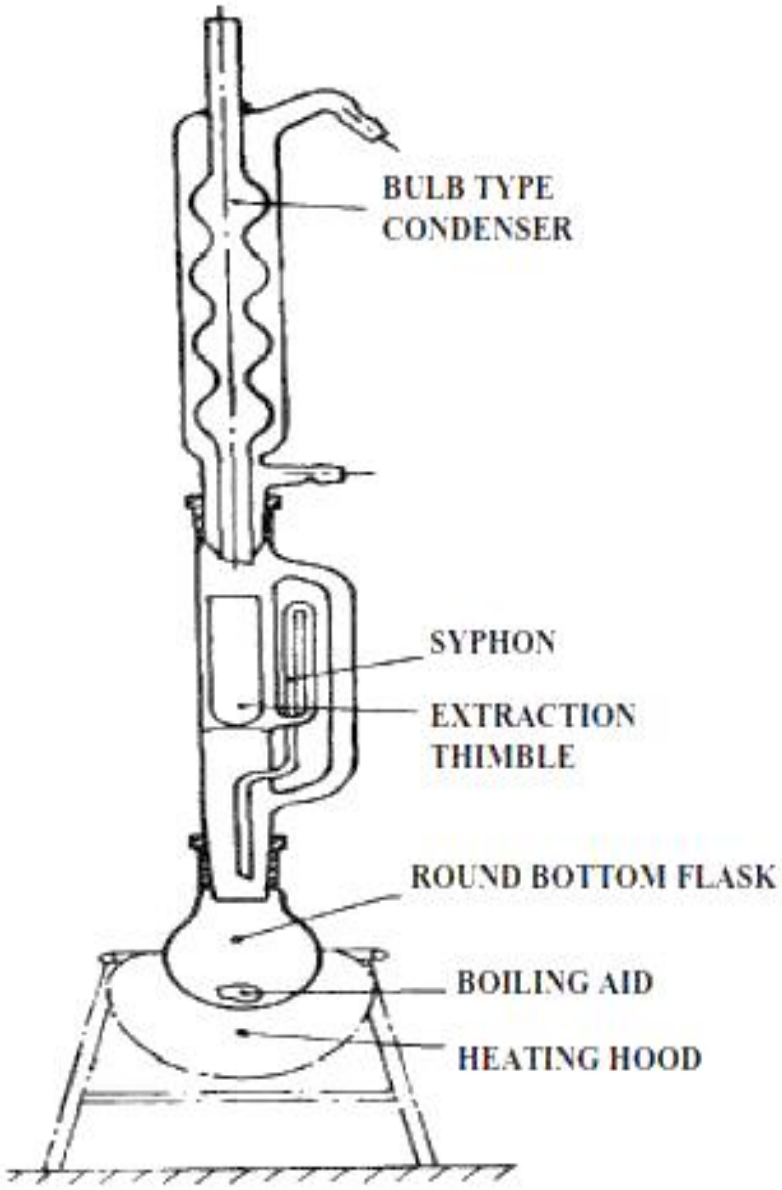

Fig 9; Soxhlet extraction assembly

4. Protein

Most natural foods contain protein. There are exactly nine amino acids which are essential for human health and must be gotten from dietary sources. **Protein found in beef and other meats product are referred to as "complete" proteins because they contain all the essential amino acids required to promote human health whereas,** Plant-based proteins are referred to as "incomplete" proteins because they do not contain the essential amino acids. From a nutrient standpoint, beef is mostly comprised of protein and contains no carbohydrates. Hence, beef is a very efficient source of protein that is bioavailable and readily metabolized by the body

The quantification of total protein in food and food products can be performed either directly or by determining total nitrogen from the conversion of crude protein using a suitable conversion factor. The protein content can be calculated from the total nitrogen determined by either Kjeldahl method or Dumas/Pregl-Dimas method. Amides (abundant in young shoots), ammonium salts, lecithin, nitrates, and meat extracts in addition to protein contain nitrogen in varying proportions. The protein calculated by a suitable conversion factor is a valuable representation of the approximate crude protein content in the sample.

Kjeldahl Method of protein determination

This Kjeldahl method is widely accepted method used for protein determination in food and food products. The method comprises of three steps:

Digestion; Involves the decomposition of organic matter heating in the presence of concentrated sulphuric acid, the end product is ammonium sulphate solution.

Distillation; Ammonium sulphate is converted into gaseous ammonia by addition of an excess base, which is followed by boiling and condensation of the ammonia into a receiving solution (acid).

Titration; The rate of digestion and the completeness of the breakdown of nitrogenous compounds to ammonium sulphate depends on the following;

- The heating temperature
- The Amount of boiling point elevator used (alkali sulphate).
- Addition of a catalyst (mercury, copper sulphate, titanium dioxide), oxidant (hydrogen peroxide).
- Reflux rate of sulphuric acid
- Length of digestion.

  Ammonia is liberated from the acid digestion mixture by distillation in the presence of alkali (NaOH).

5. **Micronutrient (Metals)**

Ash content determination of food is essential to determine so that the full nutritional value, quality and stability of the food can be established. Ash content can affect different characteristics of food including physiochemical and nutritional properties.

Determining the ash content in food samples is part of the proximate analysis necessary for nutritional evaluation. This ensures the safety of foods, making sure there are no toxic minerals present. The ash content in food can also impact the taste, texture and stability of foods so it is vital to know the mineral content for quality control purposes.

Ash content determination of food is essential to so that the full nutritional value, quality and stability of the food can be established.

Micronutrients in food can be quantified by utilizing the ash content yield obtained after ashing the sample. An atomic absorption spectrophotometer operates on the following principle. Light from special monochromatic sources with the wavelength of the resonance line of the element to be determined is passed through a vapor of neutral atoms. The diminution of the light beam by the activated atoms is monitored by a detector and recorded by a read-out system. Because the wavelength of the light emitted from the excited atoms in the flame is identical with the wavelength of the source line, radiation from both, the lamp and the flame, is passed by the monochromator into the light detector. In order to prevent the undesired flame radiation from interfering with the measurement, the light source is modulated and the currents from the detector are fed into an a.c. amplifier. The latter will amplify only the a.c. components of the output of the detector, while d.c. currents are thus excluded from measurement. (Alfred 1964)

A recent study on the **Qualitative effects of Paracetamol** use in cooking meat carried out by a group of final year Pharmacy students of the University of Benin further showed that the degradation products of paracetamol could be extracted and obtained by successfully using ethyl acetate as a solvent. It was also inferred that Para-aminophenol is produced when paracetamol is used to cook cow beef in increased amount as boiling time progresses and Para-aminophenol is not produced when paracetamol is boiled alone in distilled water as paracetamol is stable in acid, base and water

Although, prior studies have not been extensively carried out to quantify and compare nutritional parameters of meat cooked with paracetamol, it is pertinent to ask the question of “what other

significant effect can be observed when paracetamol is used to cook meat (cow beef) for the sole purpose of tenderizing?"

JUSTIFICATION OF THE STUDY

The rationale behind this study was to quantitatively evaluate the effects of Paracetamol use in cooking cow beef by comparing the changes in some measurable quantitative parameters when beef is cooked with Paracetamol for 30 minutes.

These parameters include; Cooked Solution pH, Proximate analysis of essential compositions of the meat (moisture content, crude protein content, fat and ash content) and Metal analysis of some essential metals of which include; Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (k), Iron (Fe), and Zinc(Zn) in both beef samples.. The essence is to determine if there will be a change in parameters and how the changes will interfere with composition of the beef sample.

AIM AND OBJECTIVES OF THE STUDY

As earlier stated, this study is aimed at comparing the changes in each of the selected quantitative parameters when meat is cooked with Paracetamol

To achieve this, the following objectives would be focused on

- To quantitatively determine the cooked solution pH of the both samples
- To quantitatively determine the moisture content retained in both meat samples
- To quantitatively determine and compare the crude protein content in both meat samples using the **Kjeldahl Protein Determination Method**

- To Quantitatively determine and compare the crude fat content in both beef samples with the aid of the **Soxhlet Extraction Method**
- To quantitatively determine and compare the amount of specific Metals (Micro nutrients) of which include; Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (k), Iron (Fe), and Zinc(Zn) in both beef samples using **Atomic absorption spectroscopy**

CHAPTER TWO

2.0. MATERIALS AND METHODOLOGY

2.1. Materials

Meat (cow beef), distilled water, beakers, test tubes, n-Hexane, Digital pH tester, pure sample of paracetamol powder, weighing balance, measuring paper, Soxhlet apparatus, sodium sulfate, stainless steel pot, copper 2 sulphate pentahydrate, 250ml glass tubes, 35% sodium hydroxide, 0.1N hydrochloric acid, sodium sulfate, potassium sulfate, concentrated sulphuric acid, 1% boric acid, oven, distillation flask, graham condenser, distilled water, pipes, conical flasks, water circulator apparatus, measuring cylinder, atomic absorption spectrophotometer (Buck Scientific 210VGIP), 5% Nitric acid

2.2. Methods Of Quantitative Analysis

Approximately 1100g of cow beef was utilized for this experiment. Exactly 500g of cow beef were weighed separately into two stainless steel pots labeled A and B, 5g of pure paracetamol powder (1g for each 100g of cow beef) was added to the first pot labeled A, 300mL of distilled water was measured and added into both pots. Heat was applied at 100^{o}c and the cow beef samples were each allowed to cook for 30minutes**.**

TEST FOR pH

After 30minutes, the beef broth in both pots was collected into two 100mL beakers labeled A and B, the PHS-3C pH meter was used to determine the pH of the broth obtained from both cooked samples .The pH of samples A (with paracetamol) and B (without paracetamol) were determined by first calibrating the electrode and this was done by immersing the electrode in distilled water to achieve a pH of 7.

Each of the samples was then tested while making sure that the electrode was continuously recalibrated with distilled water as soon as each test was done.

PROXIMATE ANALYSIS

A) Determination of moisture content

Using the loss on drying method, 50g of cooked beef were each measured onto two weighing papers labeled, A (with paracetamol) and B (without paracetamol) , the samples were transferred into the oven and allowed to dry at a temperature of 80^{o} C and the dry weight was measured.

The amount of moisture was determined by subtracting the dry weight from the initial weight, and the moisture content was then calculated as the amount of water divided by the dry weight or total weight. The procedure was duplicated and the results were recorded for comparison.

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B) Determination of protein content using kjeldahl apparatus

The Kjeldahl method involves a three-step approach to the quantification of protein: digestion, distillation, and titration. The methods are as follows;

Exactly 2g of cooked beef is pounded and weighed into a 250ml glass tube, a mixture of 14g of potassium sulphate and 1.6g of copper (ii) sulphate pentahydrate is added into the tube. 30ml of concentrated sulphuric acid was added into the flask and the mixture was shaken lightly

- **Digestion;** The sample was heated at 120^{o}C for 3 hours until a clear greenish color was obtained which turns light blue after washing with 75ml of distilled water.

- **Distillation**; About, 100mL of 35% NaOH (35g of NaOH pellets in 100 mL of distilled water) was prepared and pumped into the glass flask containing the digested sample and the distillation apparatus was connected through a tube to a condenser and then into a 250mL conical flask containing 25mL of 1% boric acid and 4 drops of methyl red indicator. Heat was applied to the glass flask and distillation was allowed to last for about 5-10 minutes after which the distillate was collected into the conical flask contained
- T**itration**; Back titration was carried out as the resulting distillate was titrated against 0.1N HCl, the volume of HCl used was noted when a pinkish coloration was observed indicating that the moles of HCl equals the moles of Nitrogen. The Nitrogen content was calculated and the percentage of crude protein in the sample was calculated using 6.25 as the common conversion factor for Cow beef

The procedure was repeated once and the results were recorded for comparison.

Formula for determination of Nitrogen content

$$\%N = \frac{\text{Vol of acid (final-Initial)} \times \text{Normality of acid} \times 14.007 \times \text{dilution factor}(1/1000) \times 100}{\text{Weight of sample (g)}}$$

% Crude protein= %Nitrogen x Conversion factor of meat sample (6.25)

C) Determination of Fat content using the Soxhlet extraction apparatus

Exactly 100g of cooked cow beef was weighed on an analytical balance into two separate weighing papers, one was labeled A (with Paracetamol) and the other labeled B (without paracetamol). The samples were mashed and grind using a mortar and pestle until the samples were as homogenous as possible. The crushed samples were mixed with sodium sulfate in a 1:1

(w/w) ratio. Once the samples were thoroughly mixed, 100g of the sample was transferred into an improvised cotton thimble because of the sample weigh. The thimble was filled two-thirds to three-fourths full.

1.) Approximately 500ml 0f solvent (n-Hexane) was poured into a 1L round-bottom flask. The Soxhlet extractor connection was placed on top and the thimble with the sample was placed in the extractor fitting. An additional 250 mL of solvent was poured into the thimble compartment. The Allihn condenser was fixated on top of the Soxhlet extractor and the water flow was turned on. Cold water was allowed to flow in from the bottom outlet and out from the top outlet of the condenser with the aid of a water circulator apparatus.

2.) The solvent was allowed to vaporize by applying heat via a heating mantle at a temperature of about 69^{o}C. The heat was adjusted as necessary to achieve regular solvent flushing. The extraction was allowed to continue for approximately 5hours after which the solvent was collected in the thimble compartment and the apparatus was turned off for 24hours to ensure that sufficient extract was released into the solvent. Finally, the Soxhlet apparatus was turned on and turned off after adequately siphoning twice.

3.) The contents of the round-bottomed flask were emptied into two pre-weighed 250mL beakers labeled A and B. The beakers were placed in an oven to concentrate the sample by removing the solvent, leaving behind the extracted fat. Once all the solvent was removed, the extracted fat was weighed. Then the weight of fat extracted from the original weighed sample was calculated. The fat content for the cow meat sample was then reported as; difference between the weight of the flask before addition of the sample

and after concentration of the sample, multiplied by 100% and divided by the original weight of the sample. (Lopez, 2020)

The procedure was duplicated and the results were recorded for comparison

D.) Determination of Ash Content

About, 4g of both samples were weighed into two pre-weighed crucibles labeled A and B, the crucibles were placed in the heating furnace at a temperature of 550^{o}C for 4 hours. After which, the samples were weighed and the ash content was calculated by subtracting the initial weight of the crucible from the weight after ash content determination divided by the weight of sample and multiplied by 100 to get the percentage composition (Ishmail, 2017)

METAL ANALYSIS

After the ash content was determined, the samples were collected, digested with 5% nitric acid, filtered into two volumetric flasks, the flasks were made up to volume with 100mL of distilled water and transferred into two sample bottles for metal analysis using Atomic absorption spectroscopy using standard nitrate salt solutions and wavelengths specific to each metal of choice (AOAC methods, 2005)

CHAPTER THREE

RESULTS

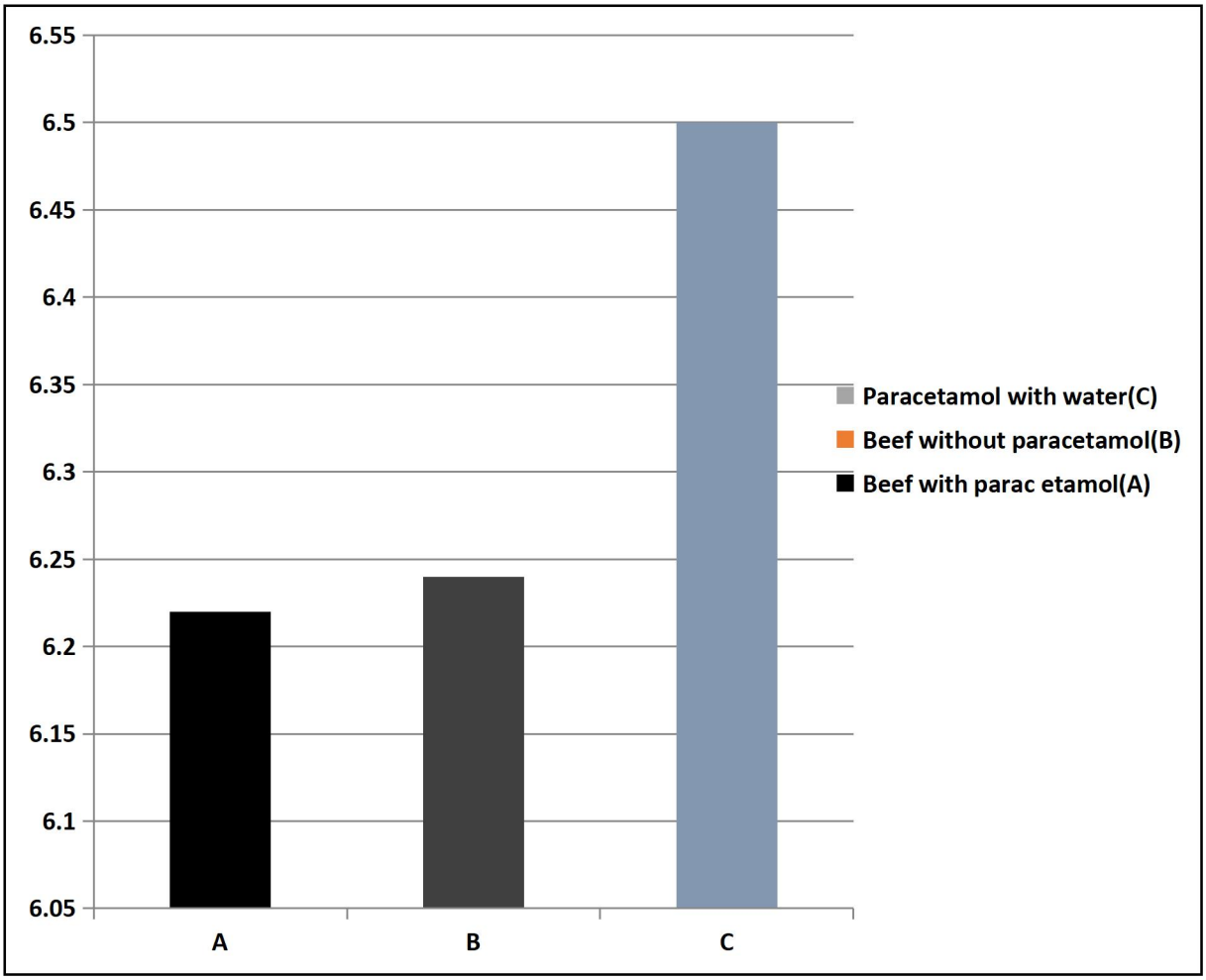

Fig 10; showing pH values for both samples compared with paracetamol and distilled water

The Physiological pH values of both samples in comparison, showed a less significant difference of 0.02. The results were compared with the ph value of

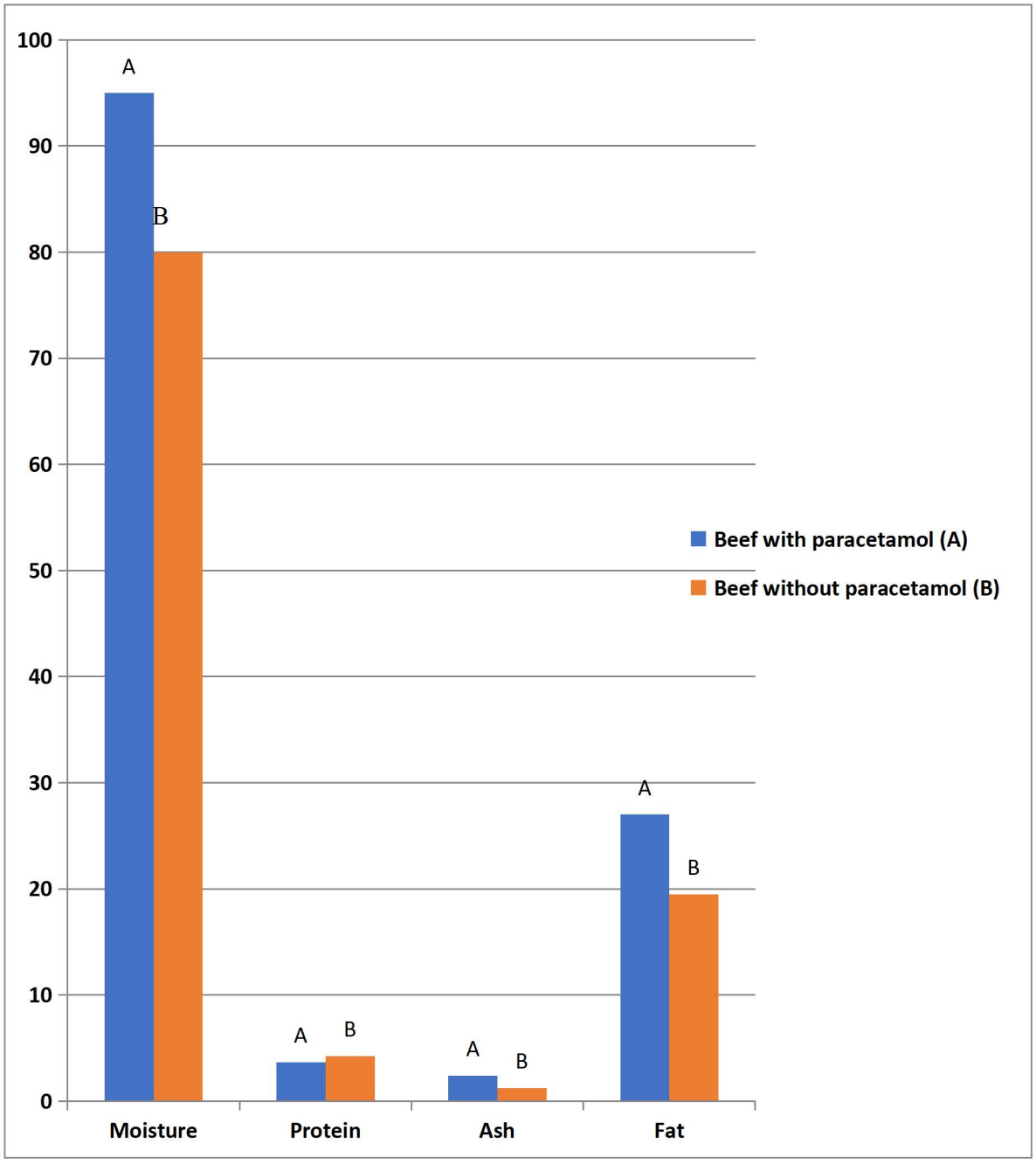

Fig11; Representing the results for proximate analysis (Appendix 1)

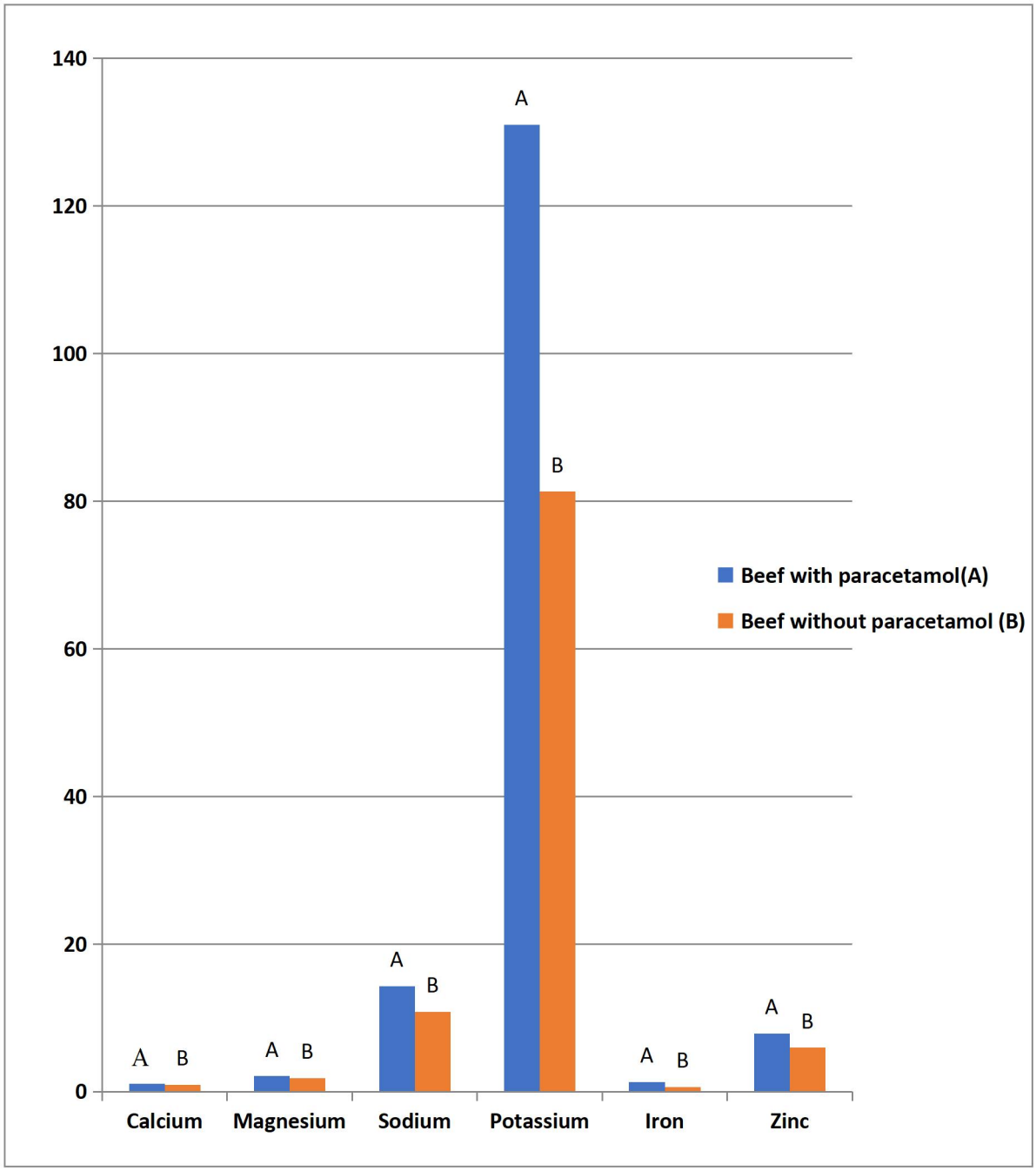

Fig 12; Representing the results for metal analysis (in Appendix 2)

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CHAPTER FOUR

DISCUSSION

Cow beef possess a host of nutritional parameters, including micro nutrients, from the representative chart obtained for pH values, both samples showed a less significant difference of 0.02 between them when compared with a heated sample of Paracetamol and distilled water. PH values are relevant determinants of the quality of meat that affects the water holding capacity (WHC), meat tenderness, color and shelf life. Paracetamol is stable in acid and base (Ahmad, 2018). When heat was applied to paracetamol and distilled water, a pH value of 6.5 was obtained on cooling. When cow beef was cooked with and without paracetamol and subjected to testing, a pH range of 6.22-6.24 (0.02) was obtained respectively. The difference in pH seen when cow beef was included in cooking must have been as a result of the quality of the meat used. Meat with a pH range of 5.71-6.90 is classified as “dark cutting” with a decreased shelf life and is solely unsuitable for vacuum packaging (Varnam, 1995). Meat with a low or more acidic pH value has more preservative property than meat with a higher pH value. The comparison of paracetamol and distilled water proved that the presence of meat conferred a slightly acidic pH value on the samples which included meat.

The table for proximate analysis above showed the different results obtained when the important parameters were tested. The parameters were selected based on their relevance to the sample of choice (cow beef) and the society.

From the result obtained for the moisture content determination of both samples, we can infer that meat cooked with paracetamol contained more moisture with a percentage composition of 95%, In microwave drying technology, research into the absorbing properties of paracetamol powder although indispensable, has shown that the absorption performance of the powder

dynamically fluctuates with a series of absorption peaks, as the water content increases or the temperature rises (Zhai, 2019). Thus inferring that the paracetamol powder has the capacity to hold more moisture and its presence in the cooked beef must have been responsible for the increased concentration of moisture content observed after drying the sample. Approximately 85% of water is bound between the thick and thin myofibrils in beef. Elevated changes in the water holding capacity (WHC) and tenderness of meat occurs when the meat product is heated, including shrinkage and hardening of tissue followed by the release of juice, which are caused by changes in the meat proteins. This considerable decrease of WHC during heating is attributable to a tightening of the myofibrillar network by heat denaturation of the proteins. Moisture content is concerned with the characteristics of food product, including its physical appearance (shape, color, etc.), texture, taste, weight (which can impact the cost) in addition to factors that affect the product's shelf-life, freshness, quality, and resistance to bacterial contamination.

Excess water in a food product can cause an increase in the rate of microbial growth, which can spoil a product before it reaches the shelves and also decrease the length of time it can stay fresh for, loyalty, and ultimately revenue and profits.

The result obtained for the crude protein content of the beef, beef cooked with paracetamol showed seemingly lower values when compared with beef cooked without paracetamol and this might have been as a result of incomplete Kjeldahl digestion, The first stage in the kjeldahl method of protein determination involves the acid digestion of the sample of choice to form ammonium sulphate which further undergoes the distillation and titration stages to yield the crude nitrogen content and crude protein content of the sample. The presence of a inorganic nitrogen in paracetamol was responsible for the incomplete kjeldahl digestion which led to the

low protein content value. Kjeldahl digestion shares mild similarities with stomach acid digestion from which it has been observed that paracetamol cannot be absorbed in the stomach

The result obtained for ash content shown that beef cooked with paracetamol yielded more ash than beef cooked without paracetamol hence indicating the presence of more metallic residues in the beef. Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food product, the most common minerals and ions, are calcium, magnesium, sodium and potassium but in smaller quantities there can also be traces of manganese, zinc, iron and others.

The result obtained for crude Fat content has shown that the beef sample cooked with paracetamol yielded an increased value when compared with the sample of beef cooked without paracetamol. Although the mechanism behind this observation is not fully understood, it can be tagged with the fat accumulative properties of paracetamol (McDanell, 1992).

Beef contains numerous micro nutrients especially metal ions, some of which are of nutritional benefits while others are not. Amongst all the metals tested which include; Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (k), Iron (Fe), and Zinc(Zn), beef cooked with paracetamol showed higher values for all the metals tested when the values were compared. The high concentration of metals in the soil from which plants are grown to feed animals may reflect on the high concentration of metals in beef. Paracetamol is known to form complexes with Zinc (Zn) and Iron (Fe) (Moamen, 2017), this might be responsible for their high concentration in sample A. Increased concentration of metals in beef cooked with paracetamol may also be a reflection of the high mineral concentration obtained when the sample was ashed in determination of the ash content. Micronutrients can be produced within the body and also obtained from diets. Dietary micronutrients have several functions and are of varying relevance

to health, Calcium (Ca) is essential for heart, muscle, and digestive system health, bone development, supports synthesis and blood function, Potassium (K) and Sodium (Na) are systemic electrolytes and are essential in co-regulating ATP. The main function of Iron (Fe) is in hemoglobin formation, hemoglobin is the red pigment of the blood which carries oxygen from lungs to other tissues in the body. Every milliliter of blood contains 0.5 milligram of iron (as a hemoglobin component), bleeding drains the body's iron reserves (Theodore, 2010), Magnesium and Zinc are responsible for tissue development and function (Godswill, 2020)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

From the result obtained from the experiment, the following conclusions can be made;

Beef cooked with Paracetamol showed less significant difference in cooked solution pH when compared with beef cooked without paracetamol

Beef cooked with paracetamol showed a higher concentration of moisture content than beef cooked without paracetamol

Beef cooked with paracetamol showed less concentration of crude protein content when compared with beef cooked without paracetamol

Beef cooked with paracetamol showed a higher concentration of fat content when compared with beef cooked without paracetamol

Beef cooked with paracetamol showed increased concentration of all the metal ions experimented in the study

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APPENDIX

TABLE 1: showing results for proximate analysis (Appendix 1)

Composition	**Beef cooked with Paracetamol (A)**	**Beef cooked without Paracetamol (B)**
Moisture	95.0±2.5	80.0±5.00

Protein	3.66±0.08	4.25±0.07
Ash	2.40±0.05	1.24±0.01
Fat	27.0±1.00	19.5±0.75

TABLE 2 : showing results for metal analysis (Appendix 2)

IONS (mg)	**Beef cooked with Paracetamol (A)**	**Beef cooked without Paracetamol (B)**
Calcium (Ca)	1.10±0.05	0.95±0.03
Magnesium (Mg)	2.10±0.05	1.85±0.08
Sodium (Na)	14.3±0.01	10.8±0.11
Potassium (K)	131±0.15	81.3±0.09
Iron (Fe)	1.30±0.10	0.60±0.05
Zinc (Zn)	7.85±0.07	5.95±0.03

Table showing the different wavelengths used in metal analysis (AAS)

Metal ions	Wavelengths (nm)
Calcium	422.7
Magnesium	285.2
Sodium	589.0
Potassium	766.5

Iron	248.3
Zinc	213.9

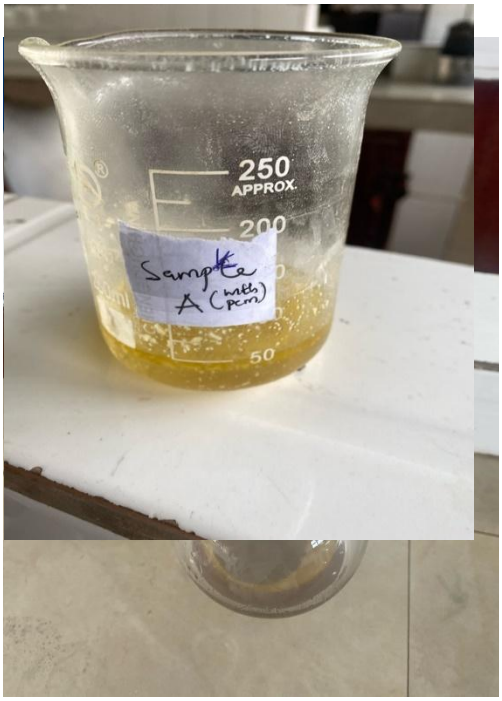
Kjeldahl distillation set up for protein determination and titration

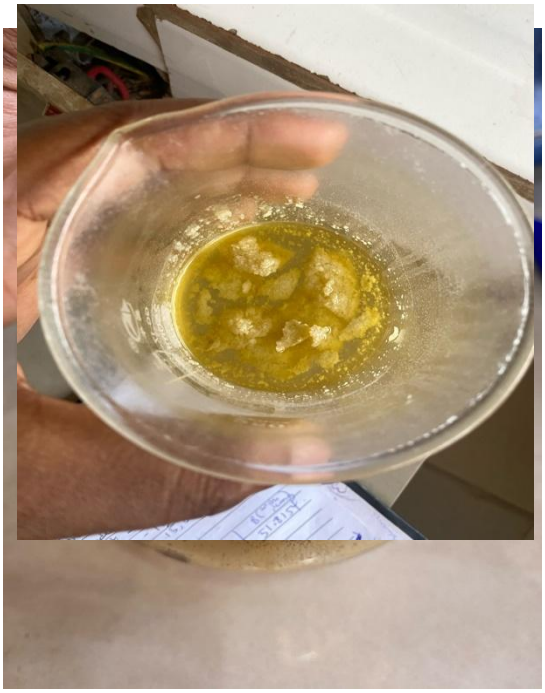
PH3C pH meter

Evidence of Ash content and sample digest for metal analysis

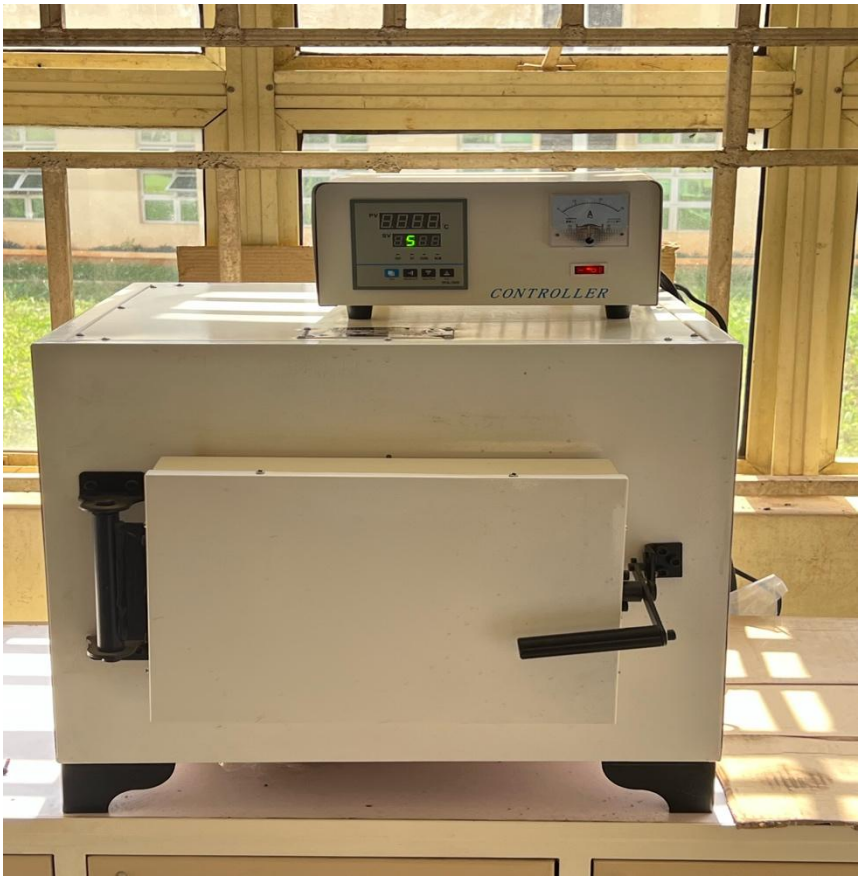
Evidence of Fat accumulation in Sample A yield (Beef cooked with paracetamol)

Soxhlet extraction set up





Sample digest for Kjeldahl protein determination



Heating Furnace

Buck scientific 210VGP atomic absorption spectrometer