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An Investigation of the Nutritional Potential of Non- Alcoholic Maheu: Study of Protein content.

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__Approval form__

The undersigned certify that they have read and recommended to the Bindura University of Science Education for acceptance; a research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Chemistry Education

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Abstract

Non-alcoholic beverages are the most favoured drinks by the majority of the people in Zimbabwe due to their high nutritional content which is also favoured by the school going children. There is an increase in the rate at which non-alcoholic beverages are being consumed in Zimbabwe. These beverages are widely consumed in rural areas in Africa. The commonly used raw materials used in the preparation of these non-alcoholic beverages are maize mealie meal, sorghum and millet. The protein content in these raw materials and non-alcoholic beverages are of paramount importance to the physical development, well-being and growth of children. These non-alcoholic beverages are a good dietary source of antioxidants which are also important in fighting against disease. The Kjeldahl method of protein content determination was used to determine the protein content in non-alcoholic beverages. The results showed that the traditional maheu with chimera contains more proteins and thus with a bit of fortification, it can be an important dietary source of proteins and antioxidants which are important for health as it contains 0.12% proteins.

Dedication

This research id dedicated to my family, Bindura staff and entire nutritionists teams within schools in the country.

Acknowledgements

I would like to thank my family, fellow chemistry teachers, chemistry tutors and laboratory technicians for advice and instructions besides the inspirational support rendered throughout this study.

To all of the above, God bless.

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CHAPTER ONE

1.1 Introduction

This chapter gives an outline of the investigation which seeks to evaluate the protein content of selected non-alcoholic beverages that are consumed in Zimbabwe. The understanding of the Nutritional composition of beverages and their contribution of the dietary patterns is important given that Zimbabwe is a developing country and is not quite capable of meeting its nutritional requirements due to factors such as climate-induced droughts, poverty and poor agricultural practices. The background of the study, statement of problem, objectives, significance of the study are covered in this chapter.

1.2 Background to the study

The National Centre for Biotechnology information, (2022) describes non-alcoholic fermented liquids as those that are either totally free of alcohol or those whose alcohol content falls below 0,7 percent, Beverages are portable drinks, aside from water, that both adults and children can drink or consume in order to satisfy hydration, thirst and energy requirements.

Non-alcoholic beverages are an integral part of the dietary intake of various age groups and socio-economic backgrounds. Their wide consumption across the population of Zimbabwe makes them a significant aspect of our daily lives and gives them a crucial role in meeting the population’s nutritional needs. The taste and refreshment value of these drinks are known but their nutritional composition in particular the protein content has not been investigated.

Proteins play an important role in many physiological functions, which include the synthesis of enzymes, hormones and antibodies. They are also essential for growth repair and maintenance of the body. This makes the understanding of the protein composition of the beverages vital in order to ascertain their nutritional value. Children whose diet lacks protein and is not balanced are prone to diseases like kwashiorkor, whose effects on child growth is well documented.

This makes it imperative that the nutritional value of all the food that is commonly fed to children be ascertained. One of the common beverages that both children and adults consume at a large scale is *maheu*. Traditional *maheu* can be prepared very easily at home and it can be both alcoholic and non-alcoholic. Given that the preparation is very easy, this means that traditional *maheu* contributes sustainably to the daily nutritional needs or dietary requirements of the majority of rural communities.

The preparation of *maheu* varies according to the materials or ingredients available. It can be prepared from finger millet, malt *(chimera)*, and water or maize meal and water. The most commonly used ingredient for making *maheu* is maize since it is one if the top 3 grain crops in Zimbabwe households as well as at a commercial scale given that it is the cheapest and most readily available source of nutrients African communities

Nhlayisa (2021) posits that *maheu* is considered a very nutritious complementary food that can be consumed for energy provision as well as for growth due to its high fibre content. Fibre has several health benefits, which include lowering the level of cholesterol as well as regulating blood sugar levels, and contains other bodily chemicals that are essential for optimum growth. Chawafambira (2012) states that *maheu* is consumed by all age groups together with other traditional fermented foods such as *mutswiwa* (fermented maize porridge) and sour milk (*mukaka wakakora*). Furthermore, some vitamins in *maheu* offer many health advantages such as vitamin A, which is needed for healthy bones and a healthy immune system.

The various types of *maheu* commonly consumed in Zimbabwe, on which the investigation will be based on include Pfuko traditional without Chimera, Pfuko buttermilk, pfuko Traditional, traditional *maheu* with Chimera as well as Shumba *maheu* with flavours such as banana, passion fruit, buttercream, vanilla, strawberry and chocolate. This investigation is therefore essential in that it may provide insights into the nutritional composition of these beverages with focus on protein content. The knowledge is essential for understanding their potential contribution to the dietary requirements and consequently health of the population.

1.3 Statement of the Problem

Basing on findings by Menguistu (2013), malnutrition is one of the major public health concerns in the developing world including countries like Zimbabwe. It is the most important risk factor for the burden of diseases resulting in about 1300 000 death per year and more than half of children’s death in Africa. Ecker and Nene (2012) define malnutrition as a chronic condition which is a result of over or under consumption of any or several essential macro or micro-nutrients in contrast to an individual’s requirement, leading to a poor function of the body.

Given that, the consumption of *maheu* in Zimbabwe is widespread and that all age groups consume it. It is imperative that its nutritional information protein in particular be ascertained so that, if need be, it is transformed or modified into a full functional food by the addition of positive ingredients such as vitamins, protein and or the removal of negative ingredients. Socio-economic shifts increase in average span, the demand for improved life quality with low health care expenses has led in the global trend to foods with added health benefits which according to Nazir *et al.*, (2019) can lead to a decline in annual health care expenses by about 20%.

1.4 Objectives

The primary aim of this study will be to investigate the protein content of *maheu* consumed by the Zimbabwean populace. The specific objectives of the study are:

- To identify and select presentative samples of *maheu*.
- To determine the amount of protein in the selected types of *maheu*.
- Compare the protein content of the selected types of *maheu.*

This will provide insights into their nutritional value, which will lead to a better understanding of the beverages' dietary contribution.

1.5 Significance of the study

The study will help the researcher improve their research skills and gain in-depth knowledge of the food and beverage industry as well as widen laboratory skills related to food analysis. The researcher will also make use of the research to acquire a bachelor of Education Honours Science Education and application degree specialising in chemistry.

1.5.1 Significance to public health

The findings of the proposed investigation are expected to contribute to a reasonable understanding of the nutritional landscape in the country that would lead to health initiatives that promote dietary practises that are balanced.

1.5.2 Consumer awareness

A knowledge of the protein content of this widely consumed non-alcoholic beverage can empower consumers to make informed choices about their dietary intake, which would promote healthier beverage options.

1.5.3 Implications for industry and policymakers

The study can inform implementation beverage industry practices on policy decision whose implementation have a bearing on the formulation marketing and regulation of maheu produced at commercial level this has the potential of improving nutritional standards for such products.

1.6 Relevance to the Chemistry Curriculum

The study can provide a practical and engaging context for Ordinary and Advanced Level chemistry students to apply their theoretical knowledge of protein chemistry in the following ways:

1.6.1. Introduction to Protein Chemistry

Begin by revisiting the basics of protein structure, function, and the biochemistry of amino acids in both Ordinary and Advanced Level chemistry classes as well as discussing the significance of proteins as macronutrients in the human diet and the importance of protein analysis for assessing nutritional quality.

1.6.2 Analytical Chemistry Techniques

The study can be used to introduce students to analytical techniques commonly used to quantify protein content, such as the Biuret assay or spectrophotometric methods. This can be achieved by discussing the principles behind these techniques, including colorimetry, protein denaturation, and the formation of coloured complexes. This can then be connected to the topics covered in the chemistry curriculum, such as chemical reactions, equilibrium, and instrumental analysis.

1.6.3 Experimental Design and Data Analysis and Interpretation

The study can be used as an opportunity to engage students in designing experiments to evaluate the protein content of non-alcoholic beverages using the techniques discussed with emphasis on the importance of sample preparation, calibration standards, data presentation, analysis and interpretation in ensuring accurate results. This is followed by calculation of protein concentrations using stoichiometric relationships and drawing conclusions about the nutritional profiles of different beverages and discussion of the significance of protein as a macronutrient in human diet and its importance for health.

1.6.4 The Chemistry of Food Analysis and Nutritional Science

The study can enable the exploration of the intersection of chemistry with food analysis and nutritional science in the context of evaluating protein content analysis by highlighting the principles governing protein detection and quantification in complex matrices like beverages. Learners are able to discuss the relationships between protein intake, dietary requirements, and health outcomes as well as considering the implications of their findings on consumer choices and dietary recommendations.

In conclusion the evaluation of protein content in non-alcoholic beverages integrates with chemistry pedagogy in that teachers can engage students in practical applications of chemistry principles, which enhances their analytical skills, and broaden their understanding of the role of chemistry in assessing food composition and nutrition. The educators can create a dynamic and applied learning experience that reinforces key chemistry concepts, and promotes critical thinking in students. This hands-on approach not only enriches the curriculum but also fosters a deeper understanding of the relevance of chemistry in addressing real-world issues related to food quality and nutrition. This interdisciplinary approach fosters a deeper appreciation for the relevance of chemistry in everyday contexts and promotes scientific literacy among learners.

1.7 Rationale of the study

Despite non-alcoholic beverages being an integral part of the daily diet for many individuals in Zimbabwe their nutritional composition, in particular the protein content of *maheu* is essential for a number of reasons.

Protein are critical macronutrients, which plays a major role in human nutrition. An essential of the protein content of *maheu* will give valuable insights into their potential contribution to the overall dietary intake of proteins in Zimbabwe.

The investigation will shed light on the nutritional profile of the popular non-alcoholic beverage, *maheu*. This will provide useful information for individuals, healthcare practitioner and policymakers concerned with public health and dietary habits.

The health implications of the investigation that an analysis of the protein will lay the groundwork for understanding their potential impact on addressing the challenge of protein deficiencies and supporting overall wellness and health.

1.8 Delimitations

Editage Insights (2019) define delimitations as attributes such as sample size, geographic location, population traits etc. that characterize the boundaries and scope of the study. Non-alcoholic beverages are widespread and numerous and they are fermented using a wide variety of cereals which include sorghum, millet, *rapoko* and maize all of which contain different levels of protein content. This research will focus on *maheu* fermented from maize, which is consumed within the geographical boundaries of Zimbabwe and available to the general population through retail outlets, supermarkets of convenience stores.

The study will use a specific sample size and selection criteria using a specific laboratory analysis method for quantifying protein that is standardised and widely accepted to ensure accuracy and reliability. Furthermore, the analysis will be limited to protein content only. Other nutritional components in the family of carbohydrates, vitamins, minerals and fats will not be the primary focus of this study although they may be under consideration within a broader context of the findings.

1.9 Limitations

Determination of protein content can be done using several methods some of which are the Dumas method, direct measurement using U.V-spectroscopy, Kjelidahl method and refractive index measurements. However, in this research the Kjelidahl method will be used even though precision may be affected by the influence of beverage processing methods that may introduce variability in the measured protein content. Variability in formulations means that there could be ingredients or additives that may affect the accuracy and consistency of the protein content analysis.

1.10 Summary

The chapter introduced the research by giving an outline of the background, significance and rationale of the study. The research objectives were established and the research questions were presented. The limitations and delimitations are also outlined. The next chapter will review related literature.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

A literature review is a critical and comprehensive summary and analysis of existing research in the form of scholarly articles, books, and other sources on a specific topic or research question. This chapter will focus on literature related to protein content of non-alcoholic beverages, the health benefits of non-alcoholic beverages as well as the raw materials used during the fermentation process.

2.1 Maheu

Beverages are not a major food, but they serve as a source of energy in several countries as they provide important nutrients. *Maheu,* which is also known as *Mageu* is a fermented non-alcoholic beverage that is very common in southern African countries. Besides its regular consumption by people of different age groups, it is also used a medium for weaning infants (ldowu, Fadahunsi & Onabiyi, 2016). As postulated by Bvochora, Reed, Read and Zvauya (1999), Maheu is traditionally made from spontaneous fermentation of sorghum or maize flour as the major solid substrate in aqueous suspension. At commercial level in the South African market, the maize flour is supplemented with low proportions of wheat flour (Idowu *et al.*, 2016). According to Kaitesi, Minnaar and Duodu, (2012), sorghum as a raw material for producing Maheu is not quite popular in South Africa because of challenges relating to its processing and poor nutritional properties such as protein content and low levels of amino acids such as lysine that are generally associated with sorghum. Maheu is popular for its refreshing and hunger quenching characteristics. Besides its use as a weaning formula for infants it is also used as supplementary food for school feeding programs and is also served to guests during social gatherings (Simango, 2002; Awobusuyi *et al.,* 2021; Daji *et al.,* 2022).

2.2 The processing of Maheu

The processing of *maheu* as described by Pswarayi and Ganzela, (2019) involves the following key stages:

I. Stirring maize flour into water and cooking the mixture into a thin gelatinized porridge.
II. Cooling the porridge and adding water to make it thin enough to be a drink.
III. Adding uncooked sorghum or finger millet malt to start fermentation, which is done at room temperature for 24 to 48 hours.

There is a very close resemblance of the above process to the one used to produce the Tanzanian beverage, *togwa* (Kitabatake *et al.,* 2003; Mugula *et al.,* 2003). The process is also similar to the one used to make *minkoyo* and *chibwantu* in Zambia as postulated by Schouswa *et al.,* (2013); Phiri *et al.,* (2019). According to Awobusuyi *et al.,* (2016) and Mqakelo *et. al.,* (2021), *mageu* or *amaheu* is processed in a similar manner in South Africa; as well as motsena in Botswana, (Mqakelo *et. al.,* 2021), *amaheu* in Swaziland and *maxau* in Namibia (Mqakelo *et al.,* 2021). Other non-alcoholic beverages of not which are very common are *bode* which is common in the western and southern parts of Ethopia. There is also *munkoya,* which is commonly produced in Zambia and DRC.

2.3 Nutritional characteristics and health benefits of maize based non-alcoholic beverages

There is widespread belief that these beverages, apart from their good taste have many health benefits and are capable of preventing diseases, Muyanja; Birungi, Ahimbisimbe, Senanda and Nyamugumya, (2010). According to Titcomb, Tanumihardjo, (2019), the fermentation of maize increases the availability of minerals and liberation of phosphorus from phytate. Nyanzi Jooste, (2012) posits that the spontaneous fermentation of maize results in a reduction in amount of carbohydrates well as some non-digestable poly and oligossacharides which are present in large quantities in maize. The beverages are big sources of nutrients which include vitamins, mineral, dietary fibre and bioactive compounds that protect against oxidative stress and inflammation diseases (Mota de Carvalho, Costa, Silva, Pimentel, Fernandes, Estevez Pintado, 2018). According to Tannock, (1989), they are also associated with many capabilities and health claims and actions on digestive, endocrine, cardiovascular, immune and nervous levels. The main disadvantages of these beverages are that they have a limited shelf life which is between 3 and 5 days which is a result of over soaring or the development of an old flavour resulting from continued microbial action.

This however is not very common in commercially produced *maheu* unless their consumed beyond their prescribed "use by" date. According to Kutyauripo, Parawira, Tinofa, Kudita, Ndengu, (2009), the wide variety of microbes involved in fermenting may lead to the generation of toxic by-products, which renders them dangerous. Another challenge as posited by Nyanzi and Jooste, (2012) there have been reports that maize has anti-nutritional factors that can interconnect with proteins, vitamins and minerals to form stable complexes, which limits their bio-availability. Such factors result in malnutrition and diminished growth as they promote poor digestibility of protein and restricting mineral bioavailability. It is against the background that the protein content of locally and commercially produced *maheu* needs to be ascertained so that appropriate measures, which may include fortification or supplementation with protein and other plant sources, can be considered.

2.4 Raw materials used in the fermentation of Maheu

Nout, (2009); Bultosa, Hamaker and BeMiller, (2008); Jideani, Jideani, (2011) all point out that throughout the African continent, cereal grains, such as maize (*Zea mays*), sorghum (*Sorghum vulgare*) and rice (*Oryza sativa*), as well as various minor grains, such as the millets, mainly pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), fonio (*Digitaria exilis-acha and D. iburua-iburu*) and teff (*Eragrostis teff)*, are of great importance. Sorghum, finger malt and *maheu* are the most commonly used grains used in making *maheu* in Zimbabwe.

2.4.1 Sorghum

Sorghum is used in the preparation of *maheu* with *Chimera*. It is a genus of about 25 species of flowering plants *poaceae* grass family. Some of the species are grown as cereals for human consumption while some of them are grown as animal fodder and cultivation is done in warm climates the world over or in naturalised pasturelands.

Fig 1.1a: sorghum plants

Fig 1.1b: sorghum grains

2.4.2 Finger millet (eleusine coracan)

Finger millet, (*eleusine coracan)* is an annual grass of the *poaceae* family, which is grown for its grain that can be used as food or for brewing. It is a robust tillering grass tufts and has erect light green stems.

Fig 1.2 a: finger malt plant

Fig 1.2 b: finger malt

2.4.3 Maize (Zea mays)

Maize is one of the most important cereal crops in the world's agricultural economy, which is used as food and as feedstock for industrial processes. There is an assertion from Brockway, Dendy and Dobraszczyk, (2001); Sangwan, Kumar and Goyal (2014) that maize has a high energy value and is utilised as human food, animal feed and fuel; it is very rich in dietary fibre and deficient in protein, with low fat and sodium contents. It usually has a very high yield potential and ranks first amongst cereals followed by rice, wheat and millet.

Fig 1.3 a: Maize plants

Fig 1.3 b: Maize cobs and grains

2.5 Current and Future Research on Maize-Based Non-Alcoholic Fermented Beverages

There is need for further research in the supplementation or fortification of non-alcoholic fermented cereal gruels and beverages since fortification or supplementation of non-alcoholic fermented beverages with protein and other plant sources is beneficial and can be a feasible and sustainable project if it is done on a commercial scale employing appropriate technologies. There is need to upgrade the technology as a way of improving or boosting measures related to food safety and the shelf life/stability of the beverages in whatever part of society they are consumed. A number of methods have been proposed to increase the manufacturing, convenience, attainability and consumption of non-alcoholic beverages that are enriched with phytochemicals which help in the resistance against fungi, bacteria and consumption by insects. Chaves-López, Rossi, Maggio, Paparella and Serio (2020); Aadil, Roobab, Sahar, Rahman and Khalil (2019) are of the opinion that the supplementing involves adding non-gluten flours or powders such as legumes and pseudocereals such as amaranths, fruits, vegetables and herbs to the cereal flours in order to enhance the quality characteristics of beverages. Furthermore, the fortification of cereal flours with legume flour can enhance protein quality. According to McGovern, Zhang. J, Tang, Zhang. Z, Hall, Moreau, Nuñez, Butrym, Richards and Wang (2004); Kedia, Wang, Patel and Pandiella (2007), phytochemicals available in fruits and vegetables could be an excellent source of probiotics, prebiotics, and synbiotics such as in non-alcoholic fermented cereal beverages.

2.6 Summary

The literature review has provided a comprehensive overview of the existing research that is relevant to the analysis of protein content in non-alcoholic beverages. The review highlighted the importance of accurate protein quantification in assessing the nutritional quality of beverages and its implications for consumer health.

Furthermore, the review underscored the significance of understanding the composition of non-alcoholic beverages, particularly in terms of protein content, as proteins are essential macronutrients that influence the overall nutritional profile of these products. By evaluating the protein levels in selected beverages, researchers can contribute valuable insights into the dietary aspects of these commonly consumed drinks. Moreover, the review identified gaps in the current literature, such as the need for more studies focusing on specific types of non-alcoholic beverages or the comparative analysis of protein content across different brands or production methods. The next chapter will look at the research methodology.

CHAPTER 3: METHODOLOGY.

3.0: Introduction.

This chapter will focus on all the analytical procedures, solution, reagents and sample preparation as well as the apparatus, instruments and reagents to be used in carrying out the experiments. In this research, the researcher is going to conduct a quantitative research in an attempt to determine the protein content in different brands of non-alcoholic beverages. The researcher will design and carry out experiments to determine the protein content in different brands of non-alcoholic fermented *Maheu* at The Heritage High School in Harare.

3.1: Chemicals and materials.

The chemicals to be used during the experimentation process to determine the protein content of non-alcoholic fermented beverages include:

- Potassium sulphate, and Se tablets.
- Sulphuric acid.
- 35% NaOH
- 4.0% Boric acid
- 0.1M hydrochloric acid.
- Methyl orange.
- Burette.
- Digestion tubes.

3.2 Percentage purity and name of manufacturers.

Table 3.1: *Percentage purities of chemicals used.*

Chemical	Percentage purity	Name of manufacturer.
Sulphuric acid, H_2SO_4	98%	Glassworld.
Potassium sulphate, K_2SO_4	99%	Glassworld.
Sodium Hydroxide, NaOH	98%	Glassworld.
Copper Sulphate, $CuSO_4.5H_2O$	99.5%	Glassworld.
Hydrochloric acid, HCl	32%	Skylabsins.
Boric acid.	99.5%	Associated Chemical Enterprise (ACE)

3.3 Sample collection.

Samples will be collected using purposive sampling method. The samples of non-alcoholic fermented *maheu* will be collected from Makokoba (Mkambo) Grains Market in Bulawayo's Oldest surbub. The samples will be include different brands of non-alcoholic fermented *maheu* and different flavours of *Shumba maheu*.

These include

- Traditional without *chimera*.
- *Pfuko* Butter milk.
- *Pfuko* traditional
- Traditional with *chimera*.

3.4 Sample preparation.

10ml of each of the collected samples of different types of *maheu* will be measured using a measuring cylinder. The samples will be digested in sulphuric acid in the presence of a catalyst which assist in the conversion of the amine nitrogen to ammonium ion. The ammonium ions are then converted to ammonia gas, heated and distilled. The ammonia gas is dissolved in a trapping solution and becomes ammonium ion once again. The trapped ammonia is then determined by the means of titration with a standard solution, and a calculation is made.

3.5 Preparation of reagents

3.5.1 Preparation of 0.1M HCl from a 32% stock solution

The law of volumetric analysis, $C_1V_1 = C_2V_2$ will be used to calculate the volume of a 32% stock solution needed to make 0.1M HCl solution in 100cm^3 as follows.

$C_1V_1 = C_2V_2$

$(10.2M)(V_1) = (0.1M)\ (100cm^3)$

$V_1 = 0.98cm^3$ of 32% HCl.

0.1M HCl will be prepared by dissolving 0.98cm^3 of a 32% stock solution of hydrochloric acid in 99.01cm^3 of distilled water.

3.5.2 Preparation of 35% sodium hydroxide solution using sodium hydroxide pellets.

A 35% NaOH solution will be prepared by dissolving 35g of NaOH pellets in 100cm^3 of distilled water.

3.5.3 Preparation of a 4% boric acid from boric acid powder.

A 4% Boric acid solution will be prepared by dissolving 4grams of boric acid powder in 100cm^3 of distilled water.

3.6 Methods of protein determination

3.6.1 The Kjeldahl method

This method was developed in 1893 by the Danish chemist, Johan Kjeldahl. The method is used determining the nitrogen content of both organic and inorganic substances like fertilisers, the soil, grains, feeds, wastewater, and foodstuffs among other substances. It is an analytical method which helps in the quantitative measurement and determination of the nitrogen present in both organic and inorganic substances.

It is widely used internationally as it is subjected to many applications requiring precision and reproducibility. Although it is unable to measure the true protein content it has the major advantage that it is the standard method of comparison against all the other methods. This method will be used based on its universality, high precision and reproducibility which all have made it the major method for the estimation of proteins in food samples. It is also a method that requires very little sample preparation and after a representative sample of the food has been selected it can usually be tested directly.

On the other hand, the various UV-visible methods require extensive sample preparation before the analysis can be carried out. These include extraction of the protein from the food into a dilute transparent solution, which usually involves time consuming homogenization, solvent extraction, filtration and centrifugation procedures. In addition, it may be difficult to completely isolate some proteins from foods because they are strongly bound to other components.

The principal basis used in the Kjeldahl technique is the oxidation of the natural organic compound using concentrated sulphuric acid. As the organic samples are oxidised the carbon it incorporates is transformed to carbon dioxide and additionally the hydrogen is converted to water. The nitrogen, from the amine group which is found in peptide bonds of the polypeptide chain is converted to NH_4^+ ions which then dissolve within the oxidizing solution and can later be converted to ammonia gas. The method therefore consists of three steps, which are digestion of the sample, distillation and titration, these steps have to be carried out in sequence to determine the amount of protein present in different samples.

3.6.1 Apparatus and reagents.

Potassium sulphate, (K_2SO_4), Selenium (Se) tablets, concentrated sulphuric acid (H_2SO_4), sodium hydroxide (NaOH) for each digestion tube, boric acid (4%), 0.1M Hydrochloric acid, Methyl orange indicator, droppers, burettes, digestion tubes, volumetric flasks, small beakers, 250ml beaker, 10ml syringes, droppers, test tubes, test tube racks, traditional *maheu* with *chimera*, traditional *maheu* without *chimera*, *pfuko* butter milk and *pfuko* traditional.

3.6.2 Digestion of the samples.

Measure at least 10.0ml of each sample of the four types of *maheu* using different measuring cylinders and pour them into four different digestion tubes clearly labelled PBM (*pfuko* butter milk), PTM (*pfuko* traditional), TMC (traditional *maheu* with *chimera*) and TM (traditional *maheu* without *chimera*). To each of the samples add 2grams of potassium sulphate followed by 0.35grams of copper (II) sulphate crystals as a catalyst. Using a clean measuring cylinder measure 10.0ml of concentrated sulphuric acid 98% and add to each of the digestion tubes. Heat the samples for 60 minutes at 430 degrees Celsius. Do not inhale the gases evolved. After heating, allow the digestion mixture cool to 50-60 degrees Celsius and add to each digestion tube 50.0ml of distilled water. Allow the digestion tubes to stand in air to cool, cold water may break the digestion tubes. The acid breaks down the organic samples via oxidation and reduced nitrogen in the form ammonium sulphate is liberated. The potassium sulphate increases the boiling point of the samples. Observe a clear and colourless solution when the digestion is complete.

$$\text{Organic sample} + H_2SO_4 = [\text{digest}]Cu^{2+}\ (NH4)_2SO_4$$

3.6.3 Distillation of the samples.

Pour the digestion samples in digestion tubes to four different distillation units and add 5.0ml of 35% sodium hydroxide solution to each of the distillation unit and distil the samples until 100ml of distillates are collected in 25.0ml of 4.0% boric acid. The sodium hydroxide solution is added to convert the ammonium salt to ammonia.

$$(NH4)_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O + 2NH_3$$

3.6.4 Titration of the samples.

Use a pipette to deliver exactly 5mls of the *pfuko* traditional *maheu* sample into a clean conical flask. Add few drops of methyl orange indicator to the sample in a conical flask. Wash the burette with little hydrochloric acid solution and allow the acid to run into the tip of the burette. Record the initial reading on the burette. Run the hydrochloric acid solution from the burette dropwise while swirling the conical flask. Stop when the solution turns to a violet colour. Record the final reading on the burette and calculate the volume of the acid used to neutralise the sample. Repeat the procedure to obtain the average titre value. Repeat the procedure using the other samples of *pfuko* butter milk *maheu*, traditional *maheu* with and without *chimera* samples and obtain the average titre value of the acid needed to neutralise the samples.

3.6.7 Post lab notes.

- During the digestion process the acid breaks down the organic samples via the oxidation process and reduce the nitrogen in the form of ammonium sulphate.
- The ammonium sulphate is added to increase the boiling point of the sample.
- The addition of the salt also increases the rate of decomposition of the digestion mixture.
- The ammonia is bound to the boric acid in the form of ammonium borate complex.
- Back titration will be used during the titration process. Back titration is a titration method where the concentration of an analyte is determined by reacting it with a known amount of excess reagent.
- Excess sodium hydroxide solution is added to the sample prior to distillation to neutralise the remaining acid and release the ammonia formed as the nitrogen-containing compounds are broken down.
- Both sodium sulphate and potassium sulphate can be used to elevate the boiling points of sulphuric acid during the digestion of the samples.
- Copper sulphate acts as a catalyst for the reaction.

3.7.0: Results for Kjeldahl method for protein content determination.

3.7.1: Results for titration of samples with HCl.

Titration results for each sample are going to be tabulated as follows:

Table 3.2: Results for the titration samples with 0.1M HCl

	Trial 1	**Trial 2**
Volume of sample solution titrated.		
Initial volume of 0.1M HCl		
Final volume of 0.1M HCl		
Volumes of 0.1M HCl that neutralised the sample.		

3.8 Calculation of the percentage of nitrogen in samples.

% nitrogen is calculated by using the following formula:

$$\frac{\text{(ml of standard acid- ml of blank) x N of acid x 1.4007}}{\text{Weight of the sample in grams.}}$$

- Where N refers to Normality (equivalent concentration of the solution)
- ml of blank refers to the millilitres of standard acid needed to titrate a reagent if boric acid is the receiving solution.

3.9 Statistical analysis of the protein content in non-alcoholic beverages.

The percentages of N in each sample will be compared and analysed using descriptive statistics.

3.10 Chapter summary

The methodology provided above is a general outline for investigating the protein content of non-alcoholic *maheu* samples. The specific details of reagents, instruments, conditions and available resources and equipment were all laid out. Relevant literature which adapts to the methodology was consulted accordingly. The next chapter will look at the results.

CHAPTER 4: RESULTS ANALYSIS.

4.0 Introduction.

The chapter will focus on the results of the experiments conducted. The results are presented in tables, graphs and figures.

4.1 Titration results of the samples with HCl

In each titration, 50ml of sample was titrated using 0.1M HCl and the results are tabulated below.

4.1.1: Results for the titration of PBM.

Table 4.1: Results for the titration PBM with 0.1M HCl

	Trial 1	**Trial 2**
Final volume of 0.1M HCl	6.8ml	13.5ml
Initial volume of 0.1M HCl	0.00ml	6.8ml
Volume of 0.1M HCl that neutralised the sample.	6.8cm3	6.7ml

Average volume used 6.75ml

4.1.2: Results for titration of TMC with 0.1M HCl

Table 4.2: Results for titration of TM with chimera with 0.1M HCl.

	Trial 1	**Trial 2**
Final volume of 0.1M HCl	14.9ml	22.9ml
Initial volume of 0.1M HCl	6.8ml	14.9ml
Volumes of 0.1M HCl that neutralised the sample.	8.1ml	8.0ml

Average volume used 8.05ml

4.1.3: Results for titration of TM with 0.1M HCl

Table 4.3: Results for titration of TM without chimera with 0.1M HCl.

	Trial 1	**Trial 2**
Final volume of 0.1M HCl	30.7ml	38.3ml
Initial volume of 0.1M HCl	22.9ml	30.7ml
Volumes of 0.1M HCl that neutralised the sample.	7.8ml	7.6ml

Average volume used 7.7ml

4.1.4: Results for titration of PTM with 0.1M HCl

Table 4.4: Results for the titration of PTM with 0.1M HCl.

	Trial 1	**Trial 2**
Initial volume of 0.1M HCl	0.00ml	6.2ml
Final volume of 0.1M HCl	6.2ml	12.3ml
Volumes of 0.1M HCl that neutralised the sample.	6.2ml	6.1ml

Average volume used 6.15ml

4.2: Determination of the protein content in the samples.

- *Calculation of the % of Nitrogen content in PTM.*

$$= \frac{(6.15ml) \times (0.1M) \times (1.4007)}{9.8g}$$

= 0.0879%

- *Calculation of the % of Nitrogen content in PBM.*

$$= \frac{(6.75ml) \times (0.1M) \times (1.4007)}{9.8g}$$

= 0.097%

- *Calculation of the % of Nitrogen content in TMC*

$$= \frac{(8.05ml) \times (0.1M) \times (1.4007)}{9.8g}$$

= 0.12%

- *Calculation of the % of Nitrogen content in TM.*

$$= \frac{(7.70cm3) \times (0.1M) \times (1.4007)}{9.8g}$$

= 0.11%

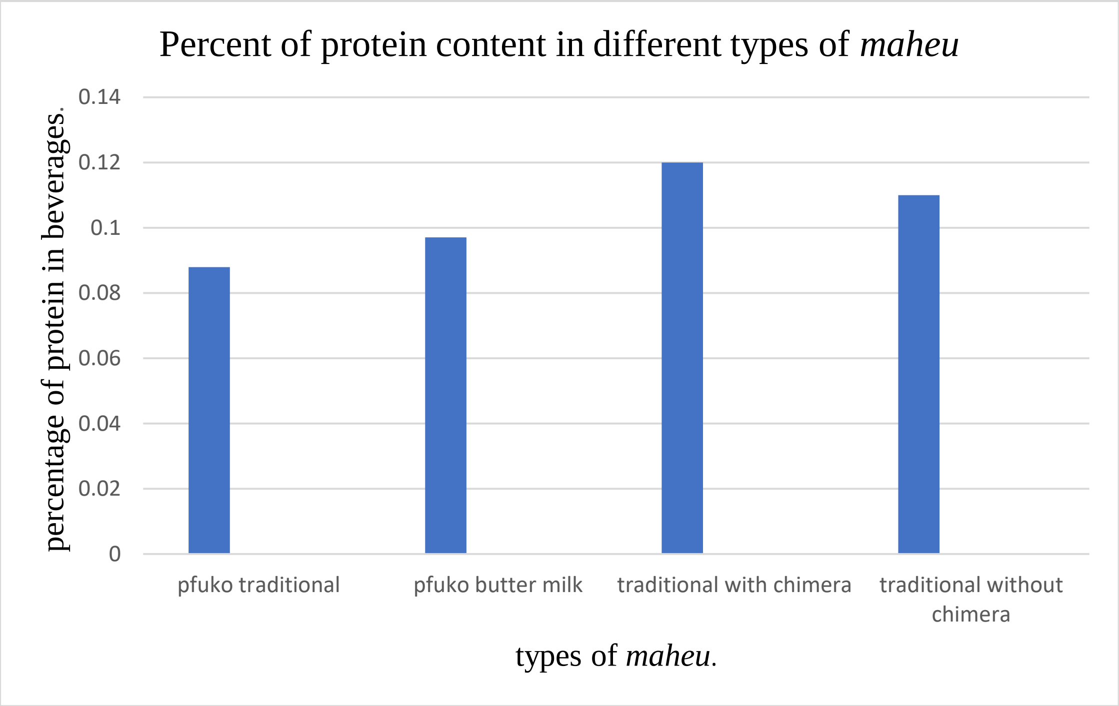
4.3: Percentage of protein content in non-alcoholic beverages.

The % protein content in non-alcoholic beverages was determined using the Kjeldahl method of nitrogen content analysis.

Table 4.5: Summary of the percentages of nitrogen content in different types of *maheu*.

Type of *maheu*	% protein content
Pfuko traditional *maheu* (PTM)	0.0879%
Pfuko butter milk *maheu* (PBM)	0.097%
Traditional *maheu* with *chimera* (TMC)	0.12%
Traditional *maheu* without *chimera* (TM)	0.11%

Fig 4.1: Percentage of nitrogen content in non-alcoholic beverages.



4..4. Summary of Chapter

This chapter presented the results of the findings on the Investigation of the Nutritional Potential of Non-Alcoholic *Maheu* with emphasis on the protein content. *Maheu* is a traditional African fermented beverage that is consumed widely across the continent. The study sought to determine the protein content of *Maheu* and evaluate its suitability as a source of dietary protein. *Maheu* samples were collected from different sources and protein extraction was performed using the Kjeldahl method. The results obtained from the protein analysis of non-alcoholic *Maheu* sheds light on its nutritional potential. The next chapter discusses the results.

CHAPTER 5: DISCUSSION.

5.0 Introduction.

The chapter will mainly focus on the outcome of the results, comparison of the protein content of the different non-alcoholic beverages as well as the recommendations to parents and the Zimbabwean population at large on nutritional non-alcoholic beverages to feed their children.

5.1 Total protein content in Maheu.

The total protein content was determined using the Kjeldahl method of protein content determination in organic food samples. During the digestion process a clear green colour indicates complete digestion of the food samples. The total protein content in non-alcoholic beverages differs due to the ingredients used during the preparation process.

Four *maheu* samples were used during the study and the ingredients used during the preparation of the samples include maize mealie-meal, sorghum, sugar and water. The millet was used as *chimera* in the preparation of traditional *maheu* with *chimera* and was found to contain more protein than the other non-alcoholic beverages. Table 4.1 shows the total protein content obtained in the four different non-alcoholic beverages. Traditional *maheu* with *chimera* was found to contain 0.12% of proteins.

Table: 5.1: Summary of Percentages of protein content

The table below shows that *Pfuko* buttermilk had 0.097% *pfuko* traditional *maheu* contained 0.0879%, traditional *maheu* with chimera contained 0.12% protein content and traditional *maheu* without *chimera* was found to contain 0.11% protein content.

Type of Maheu.	**% of protein content**
pfuko traditional *maheu*	0.0879%
pfuko butter milk	0.097%
traditional *maheu* with *chimera*	0.12%
traditional *maheu* without *chimera*	0.11%

5.2: Conclusion.

Malnutrition was observed to greatly affect a large population of young children as it affect around 149 million children under the age of five on a global scale. The shortage of essential nutrients like proteins in their diets results in unplanned weight loss, a feeling of weakness, stunted growth, swelling or fluid accumulation and this is very common in Zimbabwe. Proteins are of great importance to our health and are the fundamental constituents of the body as they are the organic constituents of protoplasm and extra-cellular materials. In a human body these proteins play a significant role as they function as enzymes, body building blocks, hormones and receptors among others.

The study conducted showed that traditional *maheu* with *chimera* contain more protein (0.12% in 10ml) than the other types of *maheu* prepared from maize-mealie or the industrially prepared *pfuko* buttermilk and the *pfuko* traditional. The Kjeldahl method which involves digestion of the samples, fractional distillation, and then titration of the samples with Hydrochloric acid was used although there are many other different method like the Dumas method, direct measurement, UV-Spectroscopy and refractive index measurement that can be used to determine the protein content in organic samples.

The analysis of the results has showed that traditional *maheu* with *chimera* are primarily important in providing proteins needed by the body. Traditional *maheu* also contain several nutrients like vitamin C, vitamin B6, folates, fibres, potassium, carbohydrates, calcium and proteins which together lower the cholesterol and decrease the risk of cardiac disease. The dietary fibre in *maheu* with malt help reduce insulin activity and increase cholesterol absorption from the gut and encourage cholesterol breakdown.

Although traditional *maheu* with malt has been observed to contain a lot of proteins, malt is actually classified as an added sugar. This means that it can be harmful if consumed in high amounts and can increase the risk of chronic conditions like diabetes, heart disease and obesity. However, nowadays the majority of adults in Zimbabwe prefer different types of *maheu* as they search for a healthier and nutritional attractive beverages compared to alcoholic beverages with very less nutritional content.

Therefore the study is of a great significance to the general public at large as it presents the benefits of traditional *maheu* with chimera with more proteins as a measure to solve the problem of malnutrition in children. The researcher therefore encourages other researchers to use other different methods to determine other nutritional composition of traditional *maheu* with chimera and also find the percentage composition of proteins in different types of malt beverages.

5.3: Recommendations.

- The researcher recommends that parents and guardians should feed their children with traditional *maheu* made from cereals like sorghum with chimera made from millet as they contain a lot of proteins as compared to other non-alcoholic beverages made from maize mealie-meal and other industrially prepared non-alcoholic beverages.
- The researcher also notes that other researchers must also find other ways of improving the methods of determining and comparing the nutrient content in different food samples.
- The researcher also recommends that other researchers must find ways through which parents can be assisted in the selection and choices of food they choose for the children as compared to other food samples with little or no benefit to the health of children.
- As the protein content is generally low in all samples, the researcher recommends that all brands need to be fortified with protein if they are to be used for dietary purposes with regard to their protein content.

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