

**DETERMINATION OF POSSIBLE CHANGES IN PROXIMATE AND MINERAL
COMPOSITION OF POWDERED MELON SEED MILK**

BY

FIDELIS GERALDINE CHIJINDU

LSC1706036

**DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
(BIOTECHNOLOGY TECHNIQUES)
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENIN CITY**

SEPTEMBER 2023

**DETERMINATION OF POSSIBLE CHANGES IN PROXIMATE AND MINERAL
COMPOSITION OF POWDERED MELON SEED MILK**

BY

FIDELIS GERALDINE CHIJINDU

LSC1706036

**A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY
TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN
CITY, EDO STATE.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF
BACHELOR OF SCIENCE DEGREE (B.Sc.), SCIENCE LABORATORY
TECHNOLOGY.**

(BIOTECHNOLOGY TECHNIQUES)

SEPTEMBER 2023

CERTIFICATION

This is to certify that the project was carried out by FIDELIS Geraldine Chijindu with Mat. No. LSC1706036 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Edo State, Nigeria.

Prof. E. O. Oshomoh

(Project Supervisor I)

Date

Mrs P. O. Omozuwa

(Project Supervisor II)

Date

Miss R. O. Osumah

(Project co-ordinator)

Date

Prof. E. O. Oshomoh

(Head of Department)

Date

External Examiner

Date

DEDICATION

This project is dedicated to Jehovah God, my caring family and my small circle of friends for their unfailing love through the stress.

ACKNOWLEDGEMENTS

First, my sincere gratitude goes to Jehovah for his constant guidance and protection throughout my stay at the University of Benin.

I would like to extend my sincere gratitude and acknowledgment to my project supervisor, Mrs. Omozuwa Precious, and Laboratory Technologist, Mr. Clinton, for their time, effort, and ever-receptive ears, which guided me every step of the way through the completion of this project.

I would like to express my deep appreciation to the Head of the Department of Science Laboratory Technology, Dr. E. O. Oshomoh, the project coordinator, Dr. Miss R. O. Osumah, and all the other lecturers in the Department of Science Laboratory Technology, for their contributions.

My special thanks go to my dear parents, Mr. and Mrs. Afakwu, my loving siblings, Fidelis Noye, Fidelis Rainny, and Fidelis Leonard, and my big sis, Okoh Ruth, for their unfailing love, words of encouragement and support.

I also want to acknowledge the support of my friends, Ziboh Lois, Ojeile Blessing, Ehimwenma Marvelous, Okiokio Gloria, Emuobonuvie Kevin, Wanogo Miracle, Yahya Kudirat, Utih Joseph, Okoeman Faith, and every other person who directly or indirectly made my Bsc. Degree in Science Laboratory Technology a success.

Thank you all.

TABLE OF CONTENTS

Certification	iii
Dedication	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	viii
List of Figures	ix
Abstract	x
Chapter One	1
1.0 Introduction	1
1.1 Statement of the Problem	1
1.2 Aim of the Study	1
1.3 Objectives of the Study	1
Chapter Two	3
Literature Review	3
2.0 Melon Seed	3
2.1 Milk	5
2.11 General Composition of Dairy Milk and Its Importance to Humans	5
2.12 Factors That Affect Milk Composition in Cows	6
2.2 Vegetable Milk And Its Health Benefits	7
2.21 Vegetable Milk	7
2.22 Health Benefits of Vegetable Milks	7
2.3 Proximate Analysis	10
2.4 Methods and Techniques for Proximate Analysis	11
2.4.1. Sample Collection and Preparation	11
2.4.2. Determination of Moisture Content	11

2.5.	Significance/Importance Of Proximate Analysis On Powdered Milk	16
2.7	Mineral Compostion Of Powdered Milk	17
2.7.1	Mineral Analysis	18
2.7.1.2	Inductively Coupled Plasma Optical Emisssion Spectroscopy (Icp – Oes)	18
2.7.2.3	Atomic Absorption Spectroscopy	19
	Chapter Three	21
	Materials and Methods	21
3.0.	Collection of Sample	21
3.1.	Materials	21
3.1.1.	Apparatus	21
3.1.2.	Equipment	21
3.1.3.	Reagents	21
3.2.	Sample Preparation	22
3.2.1.	Production of Liquid Melon Seed Milk	22
3.2.2.	Production of Powdered Melon Seed Milk	22
3.3.	Determination of Moisture Content	22
3.4	Determination of Ash Content	23
3.5	Determination of Crude Fat	23
3.6	Determination of Crude Fibre	24
3.7	Determination of Crude Protein	25
3.8	Determination of Carbohydrate	26
3.9	Mineral Analysis	26
3.9.1	Wet Ashing of Sample	26
3.9.2	Elemental Analysis	26

Chapter Four	27
Results	27
Chapter Five	34
Discussion and Conclusion	34
5.1 Discussion	34
5.2 Conclusion	35
5.3 Recommendation	36
References	37
Appendix	42

LIST OF TABLES

Table 2.1: Classification of plant-based milk alternatives based on its origin	7
Table 2.2: Main bioactive compounds of selected plant-based milk alternatives	8
Table 2.3: Mineral elements composition of the powdered milk samples	18
Table 4.1: Proximate composition of powdered melon seed milk (Month 0)	27
Table 4.2: Mineral composition of powdered melon seed milk (Month 0)	27
Table 4.3: Proximate composition of powdered melon seed milk (Month 1)	28
Table 4.4: Mineral composition of powdered melon seed milk (Month 1)	28
Table 4.5: Proximate composition of powdered melon seed milk (Month 2)	29
Table 4.6: Mineral composition of powdered melon seed milk (Month 2)	29
Table 4.7: Proximate composition of powdered melon seed milk (Month 0-2)	30
Table 4.8: Mineral composition of powdered melon seed milk (Month 0-2)	31

LIST OF FIGURES

Figure 1.1: Shelled and unshelled melon seeds	4
Figure 2.1: Automated Karl Fischer Volumetric titration unit	13
Figure 2.2: Soxhlet extractor apparatus	15
Figure 2.3: Gerber fat butyrometer	16
Figure 4.1: Powdered melon seed milk	32
Figure 4.2: Freeze dryer	33

ABSTRACT

Many individuals have lactose intolerance which means that they are unable to digest significant amounts of lactose due to the low amount of the enzyme lactase in their system. Some other individuals are vegans, hence, avoid dairy products. In this study, melon seeds flour was used to produce powdered milk and stored for 0-2 months. Proximate and mineral analysis was carried out. Results shows it contains (10.85 – 13.45%) Carbohydrate, (12.89 – 13.36%) Protein, (60.82 – 62.65%) Crude fat, (0.91 – 1.11%) Crude fibre, (2.50 – 3.18%) Ash content, and (8.88 – 9.69%) Moisture content. Mineral analysis shows it is rich in Na, K, Ca, Mg, Mn, Zn, Cu, Fe, and N. It can be consumed by individuals with or without health issues. Statistically $P>0.05$, which means there was no significant change in the quality of powdered milk over the time of storage.

CHAPTER ONE

1.0 INTRODUCTION

Melon seeds are highly nutritious and offer various essential nutrients. The nutritional composition may vary slightly through the different genera. However, in general, melon seeds are a good source of protein, healthy fats, dietary fibre, vitamins, antioxidants and minerals. The dietary fibre content in egusi seeds promotes healthy digestion, aids in maintaining bowel regularity, and supports satiety. The antioxidants, such as vitamin E and phenolic compounds, help protect the body against oxidative stress. Minerals like iron, zinc, and calcium, play vital roles in various physiological processes, including blood production, immune function, and bone health.

1.1 STATEMENT OF THE PROBLEM

Many individuals have lactose intolerance which means that they are unable to digest significant amounts of lactose due to the low amount of the enzyme lactase in their system. (Swagerty Jr *et al.*, 2002). Some other individuals are vegans, hence, avoid dairy products. Due to these facts, there is a high interest in plant derived alternatives of milk and milk products. Some of the plants through which milk are already being derived include; coconut, almond, oats, rice, soy, tiger nut, etc. However, milk can also be derived from melon seeds. (El-Bialy *et al.*, 2020).

Research has shown that melon seed milk contains no lactose and has up to 3.6% of proteins, which makes it a healthy alternative for both lactose intolerant individuals and vegans. (Bastioğlu *et al.*, 2016). However, the consumption rate of melon seed milk is still very low due to its limited shelf life even when pasteurized and refrigerated. In a research work conducted by Akubor *et al.* (2002), microbial properties of pasteurized melon seed milk was studied at different storage temperatures and it was observed that at a temperature of 30°C, the melon seed milk could only be stored safely for a day, while at a temperature of 10°C, it was able to last for two days. However, by the second day, the number of bacteria was duplicated. Hence the need for spray-dried or freeze-dried melon seed milk.

The shelf life of the melon seed milk could be limited though, due to the high water activity and pH which gives a very suitable environment for the growth of microorganisms. The process of drying will reduce the moisture content and water activity which can help to combat the problem of limited shelf life. (Bastioğlu *et al.*, 2016).

Due to its high rate of moisture removal, reduced cost, and faster processing time, spray drying or freeze drying is the most used technique for drying liquid food goods, including milk and milk products. Additionally, it makes stable and useful items possible to prepare. (Bastioğlu *et al.*, 2016).

1.2 AIM OF THE STUDY

To determine the possible changes in the proximate composition and mineral elements in powdered melon seed milk stored over a period of time.

1.3 OBJECTIVES OF THE STUDY

1. To prepare whole melon seed flour.
2. To produce powdered milk from the whole melon seed flour.
3. To determine the minerals elements in the milk sample using Atomic Adsorption Spectrophotometer (AAS).
4. To determine the possible changes in the powdered milk.

CHAPTER TWO

LITERATURE REVIEW

2.0 MELON SEED

Melon seed, commonly known as egusi in Nigeria, refers to the seeds of various species of Cucurbitaceae family plants. It is primarily derived from *Citrullus lanatus*, *Citrullus colocythis*, and *Lagenaria siceraria*. (Achigan-Dako *et al.*, 2008). Generally, though, melon seeds can be found within three genera of the Cucurbitaceae family. These include: Cucumis, Citrullus, and Cucumeropsis. “They are Cucumis, Citrullus and Cucumeropsis. The genus Cucumis includes *C. melo* L. (true melon), Citrullus includes *C. lanatus* Thunb. Matsum and Nakai (Watermelon, and Brown-seeded melon or egusi melon in Nigeria) and Cucumeropsis is represented by one species in Nigeria, *C. mannii* Naud. (syn. *C. edulis* (Hooker f.) cogn.) (White-seeded melon or Mann’s Cucumeropsis).” (Ajuru and Okoli, 2013).

The plants in the Cucurbitaceae family are dicotyledonous, with large leaves, fleshy fruits called pepo, with a leathery exocarp and numerous seeds, creeping or climbing stems, and a woody root stock. It generally grows in tropical parts of the world like Africa, and Asia. (Ajuru, 2013). The seeds of melon are oval in shape, flat and generally small, containing a white cotyledon in a thin-walled yellowish shell with a thick ring around the edge. (Oyedele *et al.*, 2018).

Melon seeds (egusi) is a popular food ingredient in many African countries, particularly Nigeria and Ghana, where it is used to prepare a variety of dishes. They are typically ground or milled into a fine powder or paste, which is then used to thicken and flavor soups, stews, and sauces. Egusi seeds add a rich, nutty flavor and contribute to the thick, creamy consistency of these dishes. Egusi-based dishes are commonly prepared with a variety of meats, fish, vegetables, and spices. Some popular dishes include Egusi Soup, Egusi Stew, and Egusi Pudding. These dishes are often served with traditional staple foods such as pounded yam, fufu, or rice. (Erhirhie and Ekene, 2013).



Figure 1.1: Shelled and unshelled melon seeds. (Source: Giwa and Akanbi, 2020).

2.1 Milk

Milk (dairy) is a nutrient-rich fluid which all female mammals secrete to feed their newborn, to enable the newborn get its complete nutritional requirements such as energy, amino acids, vitamins, essential fatty acids, inorganic elements and water. (Fox, 2008). However, milk (primarily derived from cow) also holds significant nutritional value for humans of all ages. Although the composition of milk can vary, the average milk composition is about 3.2% protein, 3.6% fat, and 4.7% lactose. (Nickerson, 1995)

2.11 General Composition of Dairy Milk and Its Importance to Humans

1. Carbohydrates

The primary carbohydrate contained in milk is lactose, a disaccharide made up of glucose and galactose. Lactose provides energy and contributes to the sweet taste of milk. It makes up about 4-5% of milk's composition. (Fox *et al.*, 2015).

2. Protein

Casein and whey proteins are the two main proteins in milk. Casein accounts for about 80% of total milk proteins, while whey proteins make up the remaining 20%. Milk proteins provide essential amino acids and plays a vital role in growth, repair and maintenance of body tissues. Protein makes up about 3.5% of milk.

3. Fats

The types of fat in milk include, phospholipids, triglycerides, and sterols. Fats provide energy, contribute to the taste and texture and aid in the absorption of fat-soluble vitamins. Cow's milk typically contains around 3-4% fat, while sheep and goat milk have higher fat content.

4. Minerals

A wide range of minerals are found in milk, these include; potassium, phosphorus, calcium, magnesium, sodium, and trace elements such as selenium and zinc. These minerals are vital for bone health, nerve transmission, and muscle function.

5. Vitamins

Milk contains a range of fat-soluble and water-soluble vitamins. Vitamins A, D, E, and K are examples of the fat-soluble vitamins. The water-soluble include, vitamin C, vitamin B

complex (including thiamine, riboflavin, niacin, vitamin B6, vitamin B12, and folate). These vitamins are essential for various physiological functions, such as immune function, vision, and energy metabolism.

6. Water

Water is the largest component of milk, typically constituting around 87-89% of its total composition. It serves as a carrier for other nutrients and provides hydration. Water in milk acts as solvent for lactose, proteins, and milk salts, and it affects properties and stability. (Fox, 2008).

2.12 Factors that affect milk composition in Cows

The nutrient contained in milk can vary based on certain factors such as:

1. Environmental effect

The amount and nutritional composition of milk produced by a cow can be affected by extremes in environmental temperature. During environmental heat stress, the amount of roughage consumed is reduced which results in decreased milk production. The milk of cows produced during cooler seasons have a greater percentage of fat and protein than that produced in warmer seasons. The difference in milk composition between seasons have an average of 0.2% protein and 0.4% fat. (Nickerson, 1995).

2. Dietary effect

In order for milk to be produced, cow absorbs a number of nutrients from its blood such as; minerals, glucose, acetate, fatty acids, and amino acids. The nutrition of the dairy cattle influences the nutrients that will be found in the blood, which in turn, affects the amount of milk produced and its composition. (Nickerson, 1995; Cunningham, 1996)

3. Influence of age

The Solid-Not-Fat (SNF) content of milk decreases with age of cow. Generally, milk production increases by 30% from the first to fifth lactation, but the percentage increase progressively decreases with age. (Laben, 1963).

2.2 Vegetable Milk and Its Health Benefits

2.21 Vegetable Milk

Vegetable milks are plant derived milks which closely resemble dairy milk in terms of physical and sensory properties. In short, “plant milks are a wide variety of the water extracts of disintegrated or dissolved vegetable materials such as pseudo-cereals, oil seeds, tubers, cereals or legumes.” (Kehinde *et al.*, 2020). The most common types of vegetable milk are soy and coconut milk, however, several others have been produced such as; rice, tiger nut, walnut, oats, groundnut and almond. (Grant and Hicks, 2018).

Table 2.1: Classification of plant-based milk alternatives based on its origin.

Origin	Plant Based Milk Alternative
Cereal based	Oats, Rice, Corn, Spelt, Rye, Kamut milk alternative
Legume based	Soybean, Peanut, Bambara groundnut, Kidney bean, Lupin, Pea, Cowpea, Chickpea milk alternative
Nut based	Almond, Cashew, Coconut, Hazelnut, Pistachio, Walnut, Tiger nut milk alternative
Oil seed based	Sesame, Flaxseed, Hemp, Sunflower milk alternative
Pseudo-cereal based	Quinoa, Teff, Amaranth milk alternative
Other based	Potato, moringa, colocynth (<i>Citrullus colocynthis</i>) seeds, cantaloupe-seeds milk alternative

(Source: Reyes-Jurado *et al.*, 2021)

In recent years, the demand for vegetable milks from consumers have greatly increased. This is due to the fact that vegetable milks generally do not contain lactose and cholesterol, hence, these milks are preferred by vegetarians, lactose intolerant individuals or individuals who are on a special diet. (Ismail, 2015).

2.22 Health Benefits of Vegetable Milks

Vegetable milks, as opposed to dairy milks, are free of dairy-related allergies, lactose, cholesterol, and have a low-calorie content, which suggests that they provide less health risks for consumers. The ratio of the sum of mono- and polyunsaturated fatty acids to saturated fatty acids in vegetable milks is noticeably higher than that of dairy milks from the

perspective of fatty acid composition, indicating a healthier profile of plant-based milks. (Kehinde *et al.*, 2020).

Plants generally contain bioactive substances which are nutritional elements which although they are not essential to human health, are very beneficial. When milk gets produced from these plants, the bioactive substances become present in the milk. (Reyes-Jurado *et al.*, 2021).

Table 2.2: Main bioactive compounds of selected plant-based milk alternatives (PBMA) and their health benefits.

PBMAs	Bioactive Compound	Health Benefits
Almond milk	α -tocopherol, arabinose, flavonoids and phytosterols	<ul style="list-style-type: none"> ●Lowers plasma LDL cholesterol level ● Decreases lipid per-oxidation ● Improves gastrointestinal health ● Prebiotic properties
Coconut milk	Lauric acid Medium chain triglycerides	<ul style="list-style-type: none"> ● Promotes brain development ● Maintains the elasticity of blood vessels ● Boosts immune system ● Decreases the LDL cholesterol and increases HDL cholesterol
Soybean milk	Isoflavones Phytosterol, α -tocopherol	<ul style="list-style-type: none"> ● Ability to bind with estrogen receptors and develop properties like the estrogenic ● Alleviate menopause symptoms ● Decreases the risk of breast, prostate and colon cancer ● Protective effect against osteoporosis and cardiovascular diseases ● Properties of lowering cholesterol ● Functions as an anti-inflammatory agent
Rice milk	Phytosterols, especially β sitosterol and γ -oryzanol	<ul style="list-style-type: none"> ● Reduction of cholesterol levels ● Lowers hypertension

		<ul style="list-style-type: none"> ● Anti-diabetic properties ● Anti-inflammatory properties ● Antioxidant activity
Cashew milk	Phytosterols, polyunsaturated fats	<ul style="list-style-type: none"> ● Lowers LDL cholesterol by inhibiting cholesterol biosynthesis
Oat milk	β -glucan and phytosterols	<ul style="list-style-type: none"> ● Delay the time of gastric emptying ● Reduction of postprandial glycemic response because of the increasing of gastrointestinal transit ● Reduction of total and LDL cholesterol ● Management of body weight and blood pressure
Sesame milk	Lignans (sesamin, sesamolin, and sesaminol)	<ul style="list-style-type: none"> ● It has hypocholesterolemic activity ● Antitumor activity ● Antiviral activity
Peanut milk	Phenolic compounds	<ul style="list-style-type: none"> ● Protective role against oxidative damage and diseases like coronary heart disease, stroke, and various cancers

(Source: Reyes-Jurado *et al.*, 2021)

Olagunju *et al.*, 2013, when analyzing nutritional values of powdered milk commonly consumed in West Africa, using Dano, Cowbell, and Nunu, found that Dano had the highest protein content ($11.7 \pm 1.77\%$), while cowbell had the lowest protein content ($8.58 \pm 0.83\%$). Dano had the most ash ($5.450.29\%$), whereas Nunu had the least ($4.390.36\%$), according to the ash content. However, some of the variables studied in the three brands of milk such as carbohydrate, and moisture did not vary.

Yadav *et al.*, 2018, analyzed the influence of spray drying technology on the proximate composition of peanut (*Arachis hypogaea* L.) milk powder. In peanut milk powder, the proportions of proteins, carbohydrates, fat, ash, and crude fiber were 27.05%, 18.22%, 45.89%, 2.86%, and 1.11%, respectively. These parameters; proteins, carbohydrate, fat, and ash levels all increased when the milk was turned into a powder by spray drying, according to the research.

Elrofaei *et al.*, 2021, studied on the physico-chemical properties of eight milk powders packed in Sudan, using Nido milk as a control. In comparison to the control, Nido sample, which had a moisture percentage of 2.25%, the local commercial milk powders ranged in moisture content from 3.2 to 6.4%. The protein values ranged from 23.37 to 26.30%, the fat contents from 26.0 to 27.21%, and the ash amounts from 3.1 to 5.3%. The lactose contents ranged between 26.50-28.63%, which was lower than that of the control, Nido, at 37.15%.

2.3 PROXIMATE ANALYSIS

Proximate analysis is an analytical technique used to determine the major components of a food or biological sample. It offers useful details regarding the sample's nutritional make-up, overall quality, and suitability for different uses. The components typically analyzed in proximate analysis include moisture, ash, protein, fat, and carbohydrates. (Sharma and Gupta, 2022).

Key components analyzed in proximate analysis

The following is an overview of what proximate analysis entails.

Moisture:

The amount of water that is contained in a sample is known as its moisture content. By drying the sample to remove the water and determining the percentage of moisture lost, the moisture content of a sample can be calculated. The stability, shelf life, and microbiological development of food products are all impacted by moisture content. Typically, procedures like oven drying, Karl Fischer titration, or moisture analyzers are used to determine it. (Mauer and Bradley Jr, 2017).

Ash:

This refers to the inorganic residue that remains after all organic material has been completely burned. It provides details on the mineral composition of a sample. By burning the sample at a high temperature and weighing the residual ash, the ash content may be calculated. It serves as a gauge for sample purity and can be used to determine whether contaminants are present. Ash determination techniques include dry ashing and muffle furnace ashing. (Harris and Marshall, 2017).

Protein:

Protein analysis determines the total amount of protein present in a sample. For evaluating the nutritional content of food and feed items, it is crucial. Protein analysis can be done in a number of ways, such as the Kjeldahl method, which determines the concentration of nitrogen first and then uses a conversion factor to determine protein content. The Bradford assay, the Dumas method, and spectrophotometric techniques are additional approaches. (Chang and Zhang, 2017).

Fat:

Fat analysis determines the amount of lipid in a sample. The energy amount, nutritional value, and sensory qualities of food products can all be evaluated using this information. Gravimetric techniques, solvent extraction (such as the Soxhlet method), and analytical procedures like gas chromatography and near-infrared spectroscopy are frequently used for fat analysis. (Ellefson, 2017).

Carbohydrates:

The total amount of carbohydrates in a sample can be determined using carbohydrate analysis. It consists of a number of different components, such as sugars, starches, dietary fiber, and other carbohydrates. Depending on the particular component of interest, there are many techniques for carbohydrate analysis, including enzymatic assays, high-performance liquid chromatography (HPLC), and gravimetric approaches. (Bemiller, 2017)

Crude fiber

Crude fiber is one of the components analyzed in proximate analysis, providing information about the dietary fiber content of a biological sample. (Sharma and Gupta, 2022).

2.4 Methods and Techniques for Proximate Analysis

2.4.1. Sample collection and preparation

Sample collection for vegetable milk starts with obtaining the nut, kernels or seeds through which the milk will be derived. In some cases where the kernels or seeds cannot be directly obtained, the fruits are first collected and prepared to extract the kernels. Yetunde and Ukpong (2015), when producing almond milk, first of all collected almond fruits, peeled and scrapped off the pulp to expose the kernel. These kernels were then dehulled before milk could be extracted from them.

Generally, to extract vegetable milk, the kernels or seeds are soaked and blended into slurry. The slurry is then filtered to separate the milk from the filtrate. (Tamuno and Monday).

2.4.2. Determination of moisture content

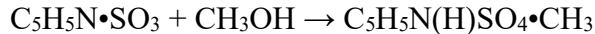
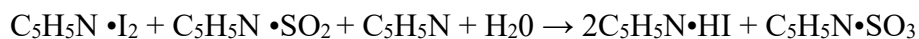
The determination of moisture content is commonly carried out through two main processes; Karl Fischer titration and drying methods such as oven drying.

Karl Fischer Titration

The Karl Fischer Titration is carried out using a Near Infrared (NIR) Spectrophotometer. The technique doesn't involve any heat and is fairly quick and accurate. The basic reaction Bunsen reported in 1853 involving the reduction of iodine by SO₂ in the presence of water is the basis for this technique:



To dissolve the iodine and SO₂, a four-component system was adjusted to add methanol and pyridine.:



These reactions show that for each mole of water, 1 mol of iodine, 1 mol of SO₂, 3 mol of pyridine, and 1 mol of methanol are used. For general work, a methanolic solution is used that contains these components in the ratio of 1 iodine:3 SO₂:10 pyridine, and at a concentration so that 3.5 mg of water = 1 ml of reagent. (Mauer and Bradley Jr, 2017).

An end point is reached when the water in the sample is consumed. Also, a constant current is detected during the titration, but this current drops at the endpoint. Based on the amount of Karl Fischer reagent that has been consumed, the amount of moisture present in the sample can be calculated. (Nagarajan *et al.*, 2006; Wüst and Rudzik, 2003; Rückold *et al.*, 2000).



Fig 2.1. Automated Karl Fischer volumetric titration unit. (Source: Mauer and Bradley Jr, 2017).

Oven Drying

In the oven drying method the sample is first weighed using an oven dried petri dish. The petri dish is oven dried to ensure that no moisture is transferred to the sample while weighing. After the weight of the sample has been recorded, the sample is transferred to an oven and allowed to dry for 2 hours at 135°C. The sample is removed from the oven and placed in a desiccator where it is allowed to cool. After cooling, it is weighed again. The moisture content is shown by the weight difference between the dry and wet states. (Bouraoui *et al.*, 1993).

Calculation

$$\% \text{Moisture content} = \frac{W_2 - W_1}{W_s} \times 100$$

Where;

W_1 = Weight of dish

W_2 = Weight of dish after drying

W_s = Weight of sample

Yadav *et al.* (2018) determined the moisture content of spray dried peanut milk powder using the oven drying method. Using a precise digital balance, five grams of spray-dried peanut milk powder was measured. The hot air oven method was used to measure the moisture content of the sample for 24 hours at 103 ± 2 °C. Then the calculation was carried out using the above formula. Using the oven drying method, Aidoo *et al.*, (2010) also determined the moisture content of peanut–cowpea milk.

2.4.3. Determination of fat content

The fat content in milk can be determined through Soxhlet extraction method and Gerber method (for liquid milk prior to powder formation).

Soxhlet extraction method

An organic solvent is used for semicontinuous fat extraction. Over the sample, the solvent condenses after being heated and volatilized. To extract the fat, the solvent drips onto the sample and soaks it. For each 15-20 minute interval, the solvent is added to the heating flask to restart the process. The sample's weight reduction or the weight of fat eliminated is used to calculate the fat content. (Carpenter, 2010). The extracted component is obtained after the solvent has been evaporated, often using a rotary evaporator. The extracted solid's insoluble component is contained in the thimble and is often discarded. (Carpenter, 2010; Nielsen, 2017).

The efficacy of solvents for the extraction of fat using Soxhlet extraction method was evaluated by Ramluckan *et al.* (2014). This showed that the solvent used in the extraction of fat using a Soxhlet extractor plays a significant role. It was concluded that “For single solvent extractions, chloroform, ethanol and hexane produced the highest lipid yields. Binary mixture of 1:1 chloro-form:ethanol showed better efficiency producing a lipid quantity of 11.76% while the best single solvent, chloroform produced 10.78%”.

Petroleum ether was used as the extraction solvent for the Soxhlet apparatus, when determining the fat content of peanut-cowpea milk powder. (Aidoo *et al.*, 2010). On the other

hand, petroleum benzene was used by Yadav *et al.* (2018) as the extraction solvent for the fat content of peanut (*Arachis hypogaea* L.) milk powder. The extraction process was carried out for two hours, then the residue was dried in an oven, cooled in a desiccator and weighed. The fat content was then calculated as follows:

$$\text{Fat content \%} = \frac{\text{Final weight of beaker along with oil} - \text{Empty weight of beaker}}{\text{Weight of sample}} \times 100$$

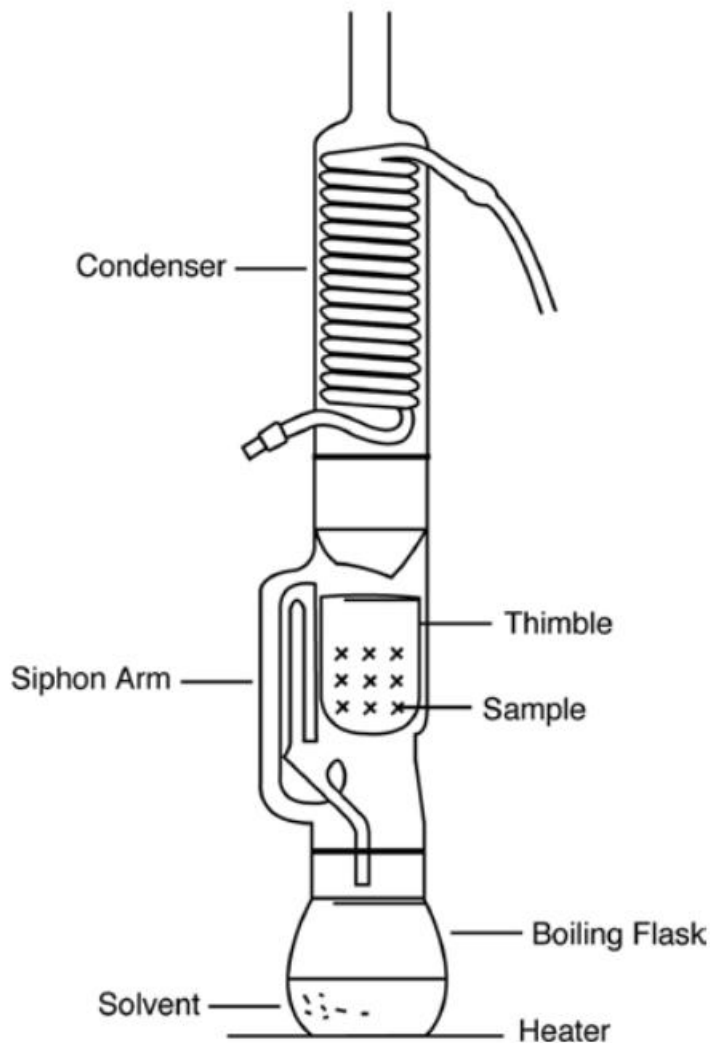


Fig 2.2. Soxhlet extractor apparatus. (Source: Ellefson, 2017).

2. Gerber method (for liquid milk prior to powder formation)

The Gerber method uses sulfuric acid, amyl alcohol, and a Gerber bottle but operates on a similar concept to the Babcock method. The sulfuric acid breaks down proteins and

carbohydrates, releases fat, and uses heat to keep the fat liquid. The Gerber test is an easy, quick, and affordable way to find out how much fat is in raw and processed liquid milk. The technique is applied globally in many different applications, such as process standardization and payment testing. In a nutshell, a sample of milk is pipetted into Gerber milk butyrometer containing sulfuric acid. The butyrometer's contents are combined after isoamyl alcohol has been added in order to break down the curd and liberate the fat. Centrifugation is used to separate the released fat in the butyrometer's neck. The calibrated scale on the butyrometer's neck is read to calculate the amount of fat in milk (g/100 g). (Kleyn, 2001).



Fig 2.3. Gerber fat butyrometer. (Source: Kleyn, 2001).

2.5. SIGNIFICANCE/IMPORTANCE OF PROXIMATE ANALYSIS ON POWDERED MILK

Nutritional Assessment:

Proximate analysis plays a critical role in assessing the nutritional composition of food, providing quantitative data on major macronutrients (protein, fat, and carbohydrates) and other essential components. This information is crucial for formulating balanced diets and making informed nutritional recommendations. (Gopalan *et al.*, 2007).

Quality Control:

Proximate analysis is essential for maintaining quality control in the food industry. By determining the composition of food products, it ensures compliance with regulatory standards and label claims, as well as consistent product quality. (Nielsen, 2017).

Formulation and Product Development:

Proximate analysis provides valuable data for the formulation and development of food products. It helps optimize formulations to meet specific nutritional targets, sensory attributes, and shelf-life requirements, allowing manufacturers to create products that meet consumer demands. (Meilgaard, 2016).

Allergen and Contaminant Detection:

Proximate analysis aids in the detection of allergens and contaminants in food, ensuring food safety. It helps identify potential allergenic ingredients and detects contaminants such as heavy metals, pesticides, and microbial pathogens. (Pomeranz, 2009).

Research and Development:

Proximate analysis serves as a foundation for scientific research in food science and nutrition. It enables researchers to investigate the impact of processing methods, storage conditions, and ingredient interactions on the nutritional composition of food, advancing knowledge in the field. (Oliveira and Manhaes, 2016).

2.7 MINERAL COMPOSITION OF POWDERED MILK

The mineral composition of powdered milk is an important factor in assessing its nutritional value and suitability for various applications. Mineral analysis provides insights into the presence and concentration of essential minerals, which play crucial roles in maintaining human health.

Commonly analyzed minerals in powdered milk include calcium, phosphorus, magnesium, potassium, sodium, and iron. These minerals contribute to various physiological functions such as bone and teeth health, nerve function, muscle contraction, and oxygen transport. The mineral content of powdered milk can vary depending on factors such as the source of milk (e.g., cow's milk, goat's milk), processing methods, and fortification practices. Different brands or types of powdered milk may have slight variations in their mineral profiles. (Ward and Legako, 2017).

2.7.1 MINERAL ANALYSIS

To obtain specific mineral analysis data for powdered milk, researchers typically employ analytical techniques such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), or inductively coupled plasma optical emission spectrometry (ICP-OES). These techniques allow for the accurate quantification of mineral concentrations in powdered milk samples. The first step in mineral analysis, however, is wet digestion or wet ashing.

Lawal *et al.*, 2015, analyzed some mineral elements in different brands of powdered milk sold in Samaru Zaria, Nigeria. These elements include calcium, magnesium, iron, copper, zinc, and manganese. The result of the analysis is summarized in the table below.

TABLE 2.3: Mineral elements composition of the powdered milk samples (mg/kg)

Sample	Ca	Mg	Fe	Cu	Zn	Mn
Brand 1	9,575	953	161	5.60	38.86	2.30
Brand 2	11,719	1040	71	5.30	41.63	9.30
Brand 3	12,157	923	88	5.40	33.84	8.30
Brand 4	15,308	1078	143	6.20	34.72	10.70
Brand 5	12,157	939	82	7.10	65.79	11.60

(Source: Lawal *et al.*, 2015)

2.7.1.2 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY (ICP – OES).

Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) is a method that uses plasma and a spectrometer to analyze the elemental makeup of samples. A peristaltic pump feeds the solution to be tested through a nebulizer and into a spray chamber. An argon plasma is created from the aerosol that was formed. In the ICP-OES, a cooled induction coil that is powered by a high frequency alternating current generates plasma at the tip of a quartz torch. As a result, a different magnetic field is created, accelerating the electrons into a circular path. Ionization, which results from the argon atom and electrons colliding, creates a stable plasma. At 6000–7000 K, the plasma is extremely hot. It can even reach 10,000 K in the induction zone. The sample is atomized, and ionized in the torch. The electrons attain a more "excited" state as a result of the thermic energy they have absorbed. When the electrons return to the ground level, energy is released in the form of light (photons). Every element

has a distinctive emission spectrum of their own that may be measured using a spectrometer. A concentration is computed using the measured light intensity at a particular wavelength and the calibration. (Radboud University, 2023)

In the determination of major and minor elements in chocolate milk sample through inductively coupled plasma optical emission spectrometry, wet digestion was first carried out. In a 125 ml conical flask, a 10.0 ml sample was transferred, along with 10.0 ml of a 1:1 HCl/H₂O solution. A watch glass was then placed on top of the flask. The sample was heated on a hot plate between 100 and 150 C for about 2 hours while gently boiling. The mixture was refluxed during this period in order to prevent drying of the mixture. The digested solution was then filtered through Whatman no. 40, 125 mm filter paper into 25.0 ml volumetric flask, then deionized water was used to make it up to the mark. After wet digestion, an ICP OES instrument was used. The blank, standard solution and sample solution were scanned in the programmed wavelength range. For each analyte peak, the background correction wavelengths were carefully chosen at the proper background placements. (Kira and Maihara, 2007).

2.7.2.3 ATOMIC ABSORPTION SPECTROSCOPY

Atomic absorption spectroscopy (AAS) is a technique used to determine the concentration of specific elements in a sample by measuring the absorption of light. Atomic absorption spectroscopy relies on the principle that atoms absorb light at specific wavelengths when they are in an excited state. The technique involves passing a beam of light through a sample containing the element of interest and measuring the amount of light absorbed by the atoms. By comparing the absorption to known standards, the concentration of the element in the sample can be determined. (Robinson, 1960).

Atomic absorption spectroscopy was used in the assessment some mineral elements in different brands of powdered milk sold in Samaru Zaria, Nigeria. For digestion, hydrogen peroxide, perchloric acid, and nitric acid were used. In Pyrex glass beakers, 0.5 g of the powdered sample was weighed, and 20 ml analytical grade HNO₃, 5 ml HClO₄, and 1 ml H₂O₂ were then added. The digestion happened as Jolanta *et al.* (1996) had predicted. The fully digested samples were allowed to cool to room temperature before being diluted with de-ionized water to a volume of 50 ml. With the help of a flame atomic absorption spectrophotometer (VARIAN 240FS, Sweden), the digested samples were examined in triplicate. For the AAS analysis, stock solutions with 1000 mg/l concentrations of Ca, Mg, Fe,

Cu, Zn, and Mn from Sigma-Aldrich were employed. These stock solutions were used to create the calibration standards for each element using the serial dilution procedure. From the calibration curves, the mineral element content of each sample was determined. Three determinations were made for each sample, and the average outcomes were reported. (Lawal *et al.*, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.0. COLLECTION OF SAMPLE

Dried and dehulled melon seeds were purchased from Ring Road market, Benin City, Edo state, Nigeria. They were then screened to remove bad ones and shaft.

3.1. MATERIALS

3.1.1. Apparatus

1. Beakers
2. Volumetric flask
3. Test tubes
4. Crucible
5. Conical flask
6. Funnel

3.1.2. Equipment

1. Muffle furnace
2. Hot air oven
3. Desiccator
4. Spectrophotometer
5. Soxhlet extractor

3.1.3. Reagents

1. Hexane
2. Sulphuric acid
3. Sodium hydroxide
4. Chloroform
5. Hydrochloric acid
6. Copper sulphate
7. Sodium Sulphate
8. Selenium
9. Sodium hypochlorite
10. Alkaline phenate
11. Sodium potassium tatarate
12. Hydrogen peroxide

13. Perchloric acid

14. Nitric acid

3.2. SAMPLE PREPARATION

3.2.1. PRODUCTION OF LIQUID MELON SEED MILK

The melon seeds were first blended using a grinding machine. All the containers to be used during the process and for storage, together with the blender, were rinsed with water which had been sterilized with Milton Sterilizing Tablets. 300g of the dry blended melon seeds was weighed and placed in a blender. 900ml of boiled distilled water was measured using a measuring cylinder and poured into the blender. The melon seeds were blended for 5 minutes and filtered using a white sieve cloth. The filtrate was collected into a bottle while the residue was blended again for a second time with 450ml of boiled distilled water, then it was filtered again using a sieve cloth. After the second filtration, the residue was collected again and blended with 225ml of boiled distilled water, then it was filtered again and discarded. Also, after each filtration, the filtrate was collected in bottles and sealed. Then it was stored in a freezer.

$$\% \text{ Yield} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100$$

3.2.2. PRODUCTION OF POWDERED MELON SEED MILK

The liquid melon seed milk was dried at Trigas Laboratory, Basic Medical Sciences, University of Benin, Benin City, Edo State, using a Freeze Dryer. The sample was brought back to the laboratory in the Department of Science Laboratory Technology, University of Benin, where it was further dried using an oven.

3.3. DETERMINATION OF MOISTURE CONTENT

Two crucibles were washed and dried in an oven and their weights were obtained using a weighing balance. 3g of the sample was weighed, placed in the weighed crucibles and dried in an oven continuously at 90°C. The sample was constantly re-weighed at 10 minutes intervals until a constant weight was obtained. The sample was cooled in a desiccator and the weight was taken. The ratio of the change in weight to the original weight expressed in percentage gives the moisture content given by

$$\% \text{ Moisture content} = \frac{W_2 - W_1}{W_s} \times 100$$

Where;

W_1 = Weight of dish

W_2 = Weight of dish after drying

W_s = Weight of sample

3.4 DETERMINATION OF ASH CONTENT

2g of the dried sample was placed into a porcelain crucible which initially was weighed and transferred into a preheated muffle furnace set at the temperature of 550°C. The furnace was left on for one hour after which the crucible with its content was transferred to a desiccator and allowed to cool. The crucible with its content was re-weighed and the weight noted. The percentage ash content was then calculated from the relationship.

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.5 DETERMINATION OF CRUDE FAT

The Pearson (1973) approach was used; this method was based on the idea that non-polar components of samples may be extracted into organic solvents with ease.

Procedure:

2g (Moist-free) of each sample, was placed into fat free thimbles. These were then weighed plugged with glass wool and introduced into Soxhlet extractors containing 160 ml petroleum ether (boiling point- 60-80°C). A clean, dry receiver flask was weighed and fitted to the extractor. The extraction unit was then assembled and cold water was allowed to circulate, while the temperature of the water bath was maintained at 60°C. Extraction was carried out for 8 h. At the end of this time, the thimble containing the sample was removed and placed in an oven at 70°C for 3 h and dried to constant weight. The weight of the Thimble and the content was then obtained using a standard analytical balance.

The difference between the weights before and after the thorough extraction was used to determine the crude fat content. Hence the percentage fat was therefore calculated as:

$$\% \text{ Fat} = \frac{X-Y}{Z}$$

where,

x = Weight sample and thimble and oil

Y = Weight of empty thimble

Z = Weight of sample

3.6 DETERMINATION OF CRUDE FIBRE

This was accomplished using the AOAC (1980) technique. Each fat-free sample was placed into a 250ml beaker and 50ml of 4% H₂SO₄ was added followed by distilled water to a volume of 200ml. This was then heated to boiling and kept boiling for exactly 30 min on a Bunsen flame, with constant stirring using a rubber-tipped glass rod to remove all particles from sides of beaker. Adding hot distilled water maintained the volume at a consistent level. After 30 min of boiling, the content was poured into a Buchner funnel fitted with an ashless Whatman no. 40 filter paper and connected to a vacuum pump. The beaker was washed several times with hot distilled water and then transferred quantitatively with a jet of hot water. Till the filtrate was acid-free as determined by litmus paper, washing was carried out on the funnel. The acid-free residue was transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200ml. The mixture was boiled for 30 min with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until it was alkaline free. Finally, the resultant residue was washed with two portions of 2ml of 95% alcohol. Filter paper residues were put into a ceramic crucible that had been previously weighed. The content of the crucible was then dried in an oven maintained at 110°C to a constant weight after cooling in a desiccator. Crucible content was then ignited in a muffle furnace at 550°C for 8hours, cooled and weighed. A duplicate determination was carried out on each sample. The percentage crude fiber was therefore calculated as:

$$\% \text{ Crude Fiber} = \frac{Y-A}{X} \times 100$$

X = Weight of sample (g)

Y = Weight of insoluble matter (g)

A = Weight of Ash (g)

3.7 DETERMINATION OF CRUDE PROTEIN

For the purpose of determining crude protein, a modified version of the micro-Kjeldahl technique was utilized, as defined by AOAC (1990).

Procedure for digestion: 1g of the sample was weighed into pre-weighed micro-Kjeldahl digestion flask together with few anti bumping granules. Each flask received two grams of the catalyst solution (CuSO₄: Na₂SO₄: SeO₂, 5:1:2 w/w), as well as 10 milliliters of nitrogen-free concentrated H₂SO₄. The flasks were placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30 min and finally at full heating (100°C) until a clear solution was obtained. For an additional 30 minutes, the simmering was maintained below the boiling point to ensure that the nitrogen was completely digested and converted to ammonium sulphate. Following completion of digestion, materials were transferred quantitatively into 100 ml volumetric flasks with washing and cooling to room temperature. Distilled water was used to adjust volumes up to the mark.

After digestion, 5ml of the filtrate from the digest was transferred with the aid of a 10ml pipette into a 25ml standard flask. 2.5ml of the Alkaline Phenate was added and the solution was agitated to thoroughly combine it. Then 1ml of Sodium Potassium Tartarate was added, shaken properly followed by the addition 2.5ml of sodium hypochlorite. After that, distilled water was used to dilute the solution to the 25ml mark, and a UV/visible spectrophotometer was used to measure the absorbance of the resulting solution at 630nm. The Nitrogen standards and the sample underwent the same processing.

CALCULATION

$\% N = \frac{\text{Instrument. Reading.} \times \text{Slope Reciprocal} \times \text{Color Vol.} \times \text{Digest Vol.}}{\text{Weight of Sample} \times \text{Aliquot Taken} \times 10000}$

$\% \text{ Crude Protein} = \% \text{ Nitrogen} \times 6.25 \text{ (AOAC, 1975)}$

3.8 DETERMINATION OF CARBOHYDRATE

By deducting the sum of the percentages of crude protein, crude fat, moisture, fiber, and ash from 100, the total amount of carbohydrates in the diet samples was determined.

3.9 MINERAL ANALYSIS

3.9.1 WET ASHING OF SAMPLE

The method described by Victor Dmitrievich (2013) was used with modification. 3g of the sample was placed in a Kjeldahl flask. 10ml of mixed acid (Nitric acid and perchloric acid mixture, ratio 3 to 1) was added to each flask. The flask and its content were mildly heated for about 20 minutes at a temperature of 40°C and then increased to about 100°C for another 40 minutes. The sample was allowed to cool, about 20ml distilled water added and filtered into a standard flask. It was then made up to the 100ml mark with distilled water

3.9.2 ELEMENTAL ANALYSIS

The elements Sodium and Potassium were assayed using Flame Photometer while Calcium, Magnesium, Iron, Copper, and Zinc were assayed using Atomic Absorption Spectrophotometer.

CHAPTER FOUR

RESULTS

Note: All values for month 0-2 are a mean of duplicate analysis \pm the standard deviation

TABLE 4.1: Proximate composition of powdered melon seed milk (Month 0)

Parameter (%)	Month 0
Carbohydrate	10.85 \pm 0.23
Crude Protein	13.36 \pm 0.40
Crude Fat	62.65 \pm 0.35
Crude Fibre	1.11 \pm 0.11
Moisture Content	8.88 \pm 0.31
Ash Content	3.18 \pm 1.92

TABLE 4.2: Mineral composition of powdered melon seed milk (Month 0)

Parameter (mg/100g)	Month 0
Sodium	446.00 \pm 1.00
Potassium	7,234.00 \pm 0.00
Calcium	745.00 \pm 5.00
Magnesium	600.00 \pm 0.00
Manganese	39.50 \pm 0.50
Zinc	61.50 \pm 0.50
Iron	60.00 \pm 0.00
Copper	3.50 \pm 0.50
Nitrogen	2.14 \pm 0.06

TABLE 4.3: Proximate composition of powdered melon seed milk (Month 1)

Parameter (%)	Month 1
Carbohydrate	13.45 ± 0.85
Crude Protein	12.89 ± 0.09
Crude Fat	60.82 ± 0.93
Crude Fiber	0.92 ± 0.01
Moisture Content	9.39 ± 0.03
Ash Content	2.54 ± 0.02

TABLE 4.4: Mineral composition of powdered melon seed milk (Month 1)

Parameter (mg/100g)	Month 1
Sodium	445.00 ± 2.00
Potassium	7208.00 ± 7.00
Calcium	752.00 ± 0.00
Magnesium	593.00 ± 3.00
Manganese	40.00 ± 0.00
Zinc	60.00 ± 0.00
Iron	59.00 ± 1.00
Copper	2.50 ± 0.50
Nitrogen	2.06 ± 0.01

TABLE 4.5: Proximate composition of powdered melon seed milk (Month 2)

Parameter (%)	Month 2
Carbohydrate	11.81 ± 0.062
Crude Protein	12.89 ± 0.0875
Crude Fat	62.21 ± 0.091
Crude Fiber	0.91 ± 0.001
Moisture Content	9.69 ± 0.012
Ash Content	2.50 ± 0.072

TABLE 4.6: Mineral composition of powdered melon seed milk (Month 2)

Parameter (mg/100g)	Month 2
Sodium	442.00 ± 0.00
Potassium	7207.00 ± 3.00
Calcium	751.00 ± 1.00
Magnesium	599.00 ± 2.00
Manganese	37.50 ± 0.50
Zinc	58.50 ± 0.50
Iron	58.00 ± 0.00
Copper	3.00 ± 0.00
Nitrogen	2.06 ± 0.014

Table 4.7: Proximate composition of powdered melon seed milk (Months 0-2)

Parameter (%)	Month 0	Month 1	Month 2	TC of whole milk powder
Carbohydrate	10.85 ± 0.23	13.45 ± 0.85	11.81 ± 0.062	36.0% - 38.5%
Crude Protein	13.36 ± 0.40	12.89 ± 0.09	12.89 ± 0.0875	24.5% - 27.0%
Crude Fat	62.65 ± 0.35	60.82 ± 0.93	62.21 ± 0.091	26.0% - 40.0%
Crude Fiber	1.11 ± 0.11	0.92 ± 0.01	0.91 ± 0.001	
Moisture Content	8.88 ± 0.31	9.39 ± 0.03	9.69 ± 0.012	2.0% - 4.5%
Ash Content	3.18 ± 1.92	2.54 ± 0.02	2.50 ± 0.072	5.5% - 6.5%

Note: The last column shows the typical composition (TC) of whole milk powder by the US Dairy Export Council. (US Dairy Export Council, 2018).

TABLE 4.8: Mineral composition of powdered melon seed milk (Months 0-2)

Parameter (mg/100g)	Month 0	Month 1	Month 2	RDA Values
Sodium	446.00 ± 1.00	445.00 ± 2.00	442.00 ± 0.00	
Potassium	7,234.00 ± 0.00	7208.00 ± 7.00	7207.00 ± 3.00	
Calcium	745.00 ± 5.00	752.00 ± 0.00	751.00 ± 1.00	800 - 1200
Magnesium	600.00 ± 0.00	593.00 ± 3.00	599.00 ± 2.00	270 - 400
Manganese	39.50 ± 0.50	40.00 ± 0.00	37.50 ± 0.50	2 - 5
Zinc	61.50 ± 0.50	60.00 ± 0.00	58.50 ± 0.50	12 - 15
Iron	60.00 ± 0.00	59.00 ± 1.00	58.00 ± 0.00	15
Copper	3.50 ± 0.50	2.50 ± 0.50	3.00 ± 0.00	1.5 – 3.0
Nitrogen	2.14 ± 0.06	2.06 ± 0.01	2.06 ± 0.014	

Note: The last column shows the Recommended Daily Allowance (RDA) of powdered milk for adults. (Lawal *et al.*, 2015).

STATISTICAL ANALYSIS:

The data generated were statistically analysed for Mean and Standard deviation and the Paired T-test was used to test the level of significance.

Fig 4.1: Powdered Melon Seed Milk.



(Photo Credit: Fidelis, G. 2023)

Fig 4.2: Freeze dryer. (Photo Credit: Fidelis, G. (2023))



(Photo credit: Fidelis, G. 2023)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

In this study, the proximate and mineral composition of powdered melon seed milk were analysed over a period of time and the complete results are shown in table 4.7 and 4.8, respectively. The carbohydrate content falls within the range of 11-13%, which is less than the typical composition (TC) of whole milk powder by the US Dairy Export Council. Low carbohydrate diet has been found to be effective for diabetic patients and people trying to lose weight. Low carbohydrate eating patterns have been proposed as a method to lower Hemoglobin A1c (HbA1c), a blood test that is used to diagnose type 2 diabetes and the requirement for antihyperglycemic drugs in individuals with Type 2 Diabetes and insulin resistance. (Landry *et al.*, 2021).

Although melon seed milk is high in fat with a range of 60-63% which is higher than the typical composition by the US Dairy Export Council at 26-40%, the fat contained in it is unsaturated fat which has been found to be the healthiest type of fat. The body cannot produce polyunsaturated fatty acids (PUFAs), hence they must be obtained from the food as they are essential fatty acids. (Abbah *et al.*, 2014).

According to Drewnowski *et al.*, 2021, most plant-based milk have a protein content that is generally lower than 1%. In contrast, the melon seed milk has a protein content of about 13% which is about half of the composition of whole milk powder as stated by the US Dairy Export Council. Also, according to Drewnowski *et al.*, 2021, most plant-based milk have a fiber content of 0.5%, however, melon seed milk contains about 1% of fiber. The melon seed milk was also found to contain about 9% and 3% of moisture and ash respectively.

P-value > 0.05, which means that there was no significant change in the quality of our freeze-dried milk parameters analyzed over a period of 0-2 months.

The result of the mineral analysis shows that melon seed milk is high in minerals such as magnesium, iron, zinc, manganese and copper, with their respective values of (593-600, 58-60, 58-62, 37-40, 2-4 mg/100g) which were significantly higher than the values of Lawal *et al.* (2015) who got (95-108, 8-16, 4-7, 0.2-1, 0.5-0.7mg/100g), respectively. However, the values of calcium were close, with that of melon seed milk being 745-752mg/100g, and Lawal *et al.* (2015) having 950-1500mg/100g.

The values of sodium and potassium of melon seed milk were also significantly higher (440-450, and 7200-7235mg/100g, respectively) when compared to Akpanyung (2006) with values of 3-12 and 1500-1710mg/100g, for sodium and potassium respectively.

5.2 CONCLUSION

This study has shown that melon seed milk is a viable option to consider within plant-based milk alternatives. This is due to its substantial protein content, including low carbohydrate content and a high proportion of unsaturated fats which aligns well with health-conscious dietary trends. The mineral composition of melon seed milk, particularly its higher levels of magnesium, iron, zinc, manganese, and copper, presents an attractive aspect from a nutritional standpoint. These minerals are essential for various physiological functions in the human body.

The overall mineral content, though varying from established values, positions melon seed milk as a source of valuable nutrients.

This study also highlights that the powdered form of melon seed milk maintains its quality attributes over a period of up to two months, as evidenced by the consistent results in the analysis. The exploration of melon seed milk as a nutritious and sustainable plant-based milk option holds promise for individuals seeking alternatives to traditional dairy while catering to their dietary needs and preferences. Further research and development in processing techniques could potentially enhance its market viability and availability.

5.3 RECOMMENDATION

Based on the comprehensive analysis of melon seed milk's composition and potential benefits, melon seed milk can be considered a valuable addition to diets for individuals with lactose intolerance or those adhering to vegan lifestyles. Its lactose-free nature and substantial protein content make it a suitable alternative to conventional dairy milk.

REFERENCES

- Abbah, O. C., Sanni, M., and Ejembi, D. O. (2014). Nutritional aspects of egusi melon–*Citrullus colocynthis* L. *Asian Journal of Science and Technology*. **5**(3), 176-180.
- Achigan-Dako, E. G., Fagbemissi, R., Avohou, H. T., Vodouhe, R. S., Coulibaly, O., and Ahanchede, A. (2008). Importance and practices of Egusi crops (*Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. cv. Aklamkpa) in sociolinguistic area in Benin. *Biotechnology, Agronomy and Society and Environment*. **12**(4): 393-403
- Aidoo, H., Sakyi-Dawson, E., Tano-Debrah, K., and Saalia, F. K. (2010). Development and characterization of dehydrated peanut–cowpea milk powder for use as a dairy milk substitute in chocolate manufacture. *Food Research International*. **43**(1): 79-85.
- Ajuru, M. G., and Okoli, B. E. (2013). The morphological characterization of the melon species in the family Cucurbitaceae Juss., and their utilization in Nigeria. *International Journal of Modern Botany*. **3**(2): 15-19.
- Akubor, P. I., Achi, O. K., Offonry, S. U. (2002) Influence of storage on chemical, microbial and consumer acceptability of a milk-like product made from melon seeds. *Plant Foods for Human Nutrition*. **57**(2): 91–196.
- Association of Official Analytical Chemists. (1980). Hortwitz, W. Official Methods of Analysis of the AOAC. 13th ed., AOAC, Washington, D.C. 858 pp.
- Association of Official Analytical Chemists (1990). Official Methods of Analysis. 15th Ed. Association of official Analytical Chemists, Washington D. C.
- Bastioğlu, A. Z., Tomruk, D., Koç, M., and Ertekin, F. K. (2016). Spray dried melon seed milk powder: physical, rheological and sensory properties. *Journal of food science and technology*. **53**(5): 2396-2404.
- BeMiller, J. N. (2017). Carbohydrate Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 333 – 359.
- Bouraoui, M., Richard, P., and Fichtali, J. (1993). A review of moisture content determination in foods using microwave oven drying. *Food research international*. **26**(1): 49-57.

- Carpenter, C. (2010). Determination of fat content. *Food analysis laboratory manual*. 29-37.
- Chang, S. K. C., and Zhang, Y. (2017). Protein Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 315 – 330.
- Cunningham, K. D., Cecava, M. J., Johnson, T. R., and Ludden, P. A. (1996). Influence of Source and Amount of Dietary Protein on Milk Yield by Cows in Early Lactation. *Journal of Dairy Science*. **79**(4): 620–630.
- Drewnowski, A., Henry, C. J., and Dwyer, J. T. (2021). Proposed Nutrient Standards for Plant-Based Beverages Intended as Milk Alternatives. *Frontiers in Nutrition*. **8**:761442.
- El-Bialy, E. F., Abd-Elkader, M. H., and Yousef, N. S. (2020). Non-Dairy Alternative Milk for People with Lactose and Casein Intolerance. *Journal of Food and Dairy Sciences*. **11**(12): 347-353.
- Ellefson, W.C. (2017). Fat Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 299-314.
- Elrofaei, N. A., Moshe, A. Y. S., Mohammed, Y. E. S., Abdalla, N. S., and Mustafa, A. A. (2021). Studies on the Physico-Chemical Properties of Milk Powder Poked in South. *International Journal Applied Science – Research and Review*. **8**(6): 22.
- Erhirhie, E. O., and Ekene, N. E. (2013). Medicinal values on *Citrullus lanatus* (watermelon): pharmacological review. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. **4**(4): 1305-1312.
- Fox, P. F. (2008). Milk: an overview. *Milk proteins*. 1-54.
- Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., and O’Mahony, J. A. (2015). Dairy Chemistry and Biochemistry. Second Edition. Springer International Publishing Switzerland.
- Giwa, S. O., and Akanbi, T. O. (2020). A review on food uses and the prospect of egusi melon for biodiesel production. *BioEnergy Research*. **13**(4): 1031-1045.
- Gopalan, C., Rama Sastri, B. V., and Balasubramanian, S. C. (2007). Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research.

- Grant, C. A., and Hicks, A. L. (2018). Comparative life cycle assessment of milk and plant-based alternatives. *Environmental Engineering Science*. **35**(11): 1235-1247.
- Harris, G.K. and Marshall, M.R. (2017). Ash Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 287 – 297.
- Ismail, M. (2015). Which is better for humans, animal milk or vegetable milk. *Journal of Nutritional Health and Food Engineering*. **2**(5): 14-15.
- Kehinde, B. A., Panghal, A., Garg, M. K., Sharma, P., & Chhikara, N. (2020). Vegetable milk as probiotic and prebiotic foods. *Advances in Food and Nutrition Research*. Academic Press. **94**: 115-160
- Kira, C. S., and Maihara, V. A. (2007). Determination of major and minor elements in dairy products through inductively coupled plasma optical emission spectrometry after wet partial digestion and neutron activation analysis. *Food chemistry*. **100**(1), 390-395.
- Kleyn, D. H., Lynch, J. M., Barbano, D. M., Bloom, M. J., Mitchell, M. W., and Collaborators: Cooper, L. S., Cusak, E., Fick, M., Hanks, T., Heslen, M. K., Johnson, J., Kleyn, D., H., Mercer, F., Monahan, D., Peat, B., and Petit M. (2001). Determination of fat in raw and processed milks by the Gerber method: collaborative study. *Journal of Association of Official Agricultural Chemists International*. **84**(5): 1499-1508.
- Laben, R. C. (1963). Factors Responsible for Variation in Milk Composition. *Journal of Dairy Science*. **46**(11): 1293–1301.
- Landry, M. J., Crimarco, A., and Christopher D. G. (2021). Benefits of Low Carbohydrate Diets: a Settled Question or Still Controversial? **10**(3): 409–422.
- Lawal, N. S., Tajuddeen, N., and Garba, B. B. (2015). Assessment of some mineral elements in different brands of powdered milk sold in Samaru Zaria, Nigeria. *International Food Research Journal*. **22**(6): 2634.
- Mauer, L. J. and Bradley Jr, R. L. (2017). Moisture and Total Solids Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 257-285.
- Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2016). *Sensory evaluation techniques* (5th ed.). CRC Press.

- Nagarajan, R., Singh, P., and Mehrotra, R. (2006). Direct determination of moisture in powder milk using near infrared spectroscopy. *Journal of Automated Methods and Management in Chemistry*. Pages 1–4.
- Nickerson, S. C. (1995). Milk production: Factors affecting milk composition. *Milk Quality*. 3–24.
- Olagunju, A., Muhammad, A., Aliyu, S., Mada, S. B., Isah, R., Abdullahi, S. A. Audu, Z. O. (2013). Nutritional values of powdered milk commercially consumed in West Africa. *International Journal of Food Nutrition and Safety*. **4**(2): 55-61.
- Oliveira, A. C., & Manhães, A. C. (2016). Food composition analysis: Importance and challenges. *Revista de Nutrição*. **29**(6): 837-849.
- Oyedele, T. A., Fatoki, O. A., Oyekanmi, J., and Kehinde, I. A. (2018). Molecular Characterization of Fungi in Stored Melon Seeds from South-West Nigeria. *Scholar Journal of Applied Science Research*. **1**(9): 20-23.
- Pearson, D. 1973. Laboratory techniques in food analysis. Butterworth and Co. Ltd. London, 31-37p.
- Pomeranz, Y., and Meloan, C. E. (2009). Food analysis: Theory and practice (3rd ed.). Springer Science & Business Media.
- Radboud University. (2023). General Instrumentation. [<https://www.ru.nl/science/gi/facilities-activities/elemental-analysis/icp-oes/>] [Accessed: 03/07/2023].
- Ramluckan, K., Moodley, K. G., and Bux, F. (2014). An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method. *Fuel*. **116**: 103-108.
- Reyes-Jurado, F., Soto-Reyes, N., Dávila-Rodríguez, M., Lorenzo-Leal, A. C., Jiménez-Munguía, M. T., Mani-López, E., and López-Malo, A. (2021). Plant-Based Milk Alternatives: Types, Processes, Benefits, and Characteristics. *Food Reviews International*. 1–32.
- Robinson, J. W. (1960). Atomic absorption spectroscopy. *Analytical Chemistry*. **32**(8): 17A-29A.

- Rückold, S., Grobecker, K. H., and Isengard, H. D. (2000). Determination of the contents of water and moisture in milk powder. *Fresenius' journal of analytical chemistry*. 368: 522-527.
- Sharma, S. and Gupta, S. (2022). Proximate Analysis: Ensure your food quality. [<https://cultivatorphytolab.com/proximate-analysis-ensure-your-food-quality/>] [Accessed: 09-06-2023].
- Swagerty Jr, D. L., Walling, A. D., and Klein, R. M. (2002). Lactose intolerance. *American family physician*. **65**(9): 1845-1851.
- Tamuno, E. N. J., and Monday, A. O. (2019). Physicochemical, mineral and sensory characteristics of cashew nut milk. *International Journal of Food Science and Biotechnology*. **4**(1): 1.
- US Dairy Export Council. (2018). Dry Whole Milk and Whole Milk Powder. [<https://www.thinkusadairy.org/products/milk-powders/milk-powder-categories/dry-whole-milk-and-whole-milk-powder>]. [Accessed: 16/08/2023].
- Ward R.E. and Legako J.F. (2017). Traditional Methods for Mineral Analysis. *Food Analysis*. Fifth Edition. (Eds: Nielsen, S. S). Springer International Publishing, Switzerland. 371-385.
- Wüst E., and Rudzik, L. (2003). The use of infrared spectroscopy in the dairy industry. *Journal of Molecular Structure*. **661–662**: 291-298.
- Yadav, P. B., Edukondalu, L., Patel, S., and Rao, D. B. (2018). Influence of spray drying technology on the proximate composition of peanut (*Arachis hypogaea* L.) milk powder. *Journal of Pharmacognosy and Phytochemistry*. **7**(5): 2852-2855.

APPENDIX

PROXIMATE COMPOSITION OF POWDERED MELON SEED MILK (DUPLICATE VALUES IN %)

S/N	Parameters	Month 0A	Month 0B	Month 1A	Month 1B	Month 2A	Month 2B
1	Carbohydrate	10.62	11.08	14.30	12.60	11.74	11.87
2	Crude Protein	13.76	12.96	12.98	12.79	12.80	12.97
3	Crude fat	63.00	62.29	59.89	61.75	62.30	62.12
4	Crude fibre	1.22	1.00	0.91	0.93	0.91	0.91
5	Moisture	9.20	8.57	9.36	9.42	9.68	9.70

MINERAL COMPOSITION OF POWDERED MELON SEED MILK (DUPLICATE VALUES IN Mg/Kg)

S/N	Parameters	Month 0A	Month 0B	Month 1A	Month 1B	Month 2A	Month 2B
1	Sodium	445.00	447.00	447.00	443.00	442.00	442.00
2	Potassium	7,234.00	7234.00	7201.00	7215.00	7210.00	7204.00
3	Calcium	750.00	740.00	752.00	752.00	750.00	752.00
4	Magnesium	600.00	600.00	596.00	590.00	601.00	597.00
5	Manganese	40.00	39.00	40.00	40.00	38.00	37.00
6	Zinc	61.00	62.00	60.00	60.00	58.00	59.00
7	Iron	60.00	60.00	58.00	60.00	58.00	58.00
8	Copper	3.00	4.00	3.00	2.00	3.00	3.00
9	Nitrogen	2.20	2.07	2.08	2.05	2.05	2.08

STATISTICAL ANALYSIS

NULL HYPOTHESIS

There is no significant difference in the quality of the proximate composition of the powdered melon seed milk and the duration (month 0-2)

Tests of Normality

QUALITY	MONTH	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
	Month 0	.391	6	.005	.686	6	.004
	Month 1	.391	6	.005	.709	6	.008
	Month 2	.399	6	.003	.693	6	.005

a. Lilliefors Significance Correction

Base on the Kolmogorov-smirnov and Shapiro wilk normality test presented in the table above, the statistical variables shows that they are not normally distributed with the Sig.(P-values) less than 0.05. Thereby non-parametric procedure can be used to analyze the data.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of QUALITY is the same across categories of MONTH.	Independent-Samples Kruskal-Wallis Test	.973	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Conclusion

Based on the null hypothesis using the the Kruskal-wallis test it was discovered that there is no significant differences in the quality of the proximate composition in the powdered melon seed milk and the duration (month 0-2), thereby retaining the null hypothesis, since the significant value (p-value) obtained was more than 0.05.

There is no significant difference in the quality of the mineral composition of the powdered melon seed milk and the duration (month 0-2)

Tests of Normality

	MONTHS	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
QUALITY	0	.436	9	.000	.490	9	.000
	1	.434	9	.000	.490	9	.000
	2	.434	9	.000	.490	9	.000

a. Lilliefors Significance Correction

Base on the Kolmogorov-smirnov and Shapiro wilk normality test presented in the table above, the statistical variables shows that they are not normally distributed with the Sig.(P-values) less than 0.05. Thereby non-parametric procedure can be used to analyze the data.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of QUALITY is the same across categories of MONTHS.	Independent-Samples Kruskal-Wallis Test	.906	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Conclusion

Based on the null hypothesis using the Kruskal-wallis test it was discovered that there is no significant differences in the quality of the proximate composition in the powdered melon seed milk and the duration (month 0-2), thereby retaining the null hypothesis, since the significant value (p-value) obtained was more than 0.05.