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Nutritional and functional values of microalgae (spirulina) naturally found in East Africa

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NUTRITIONAL AND FUNCTIONAL VALUES OF MICROALGAE (SPIRULINA) NATURALLY FOUND IN EAST AFRICA

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ABSTRACT

Sub-Saharan Africa has a higher prevalence of undernutrition, which is the underlying cause of stunting and underweight problems in children. Undernutrition is generally caused by insufficient intake of nutrients; in Africa, it's mainly due to over-reliance on plant-based staple diets which contain inadequate amount of essential nutrients. Spirulina is a naturally abundant microalgae with great nutritional values and could be utilized to fortify diets to enhance human health. Therefore, this study evaluated nutrient content, bioavailability and safety of locally found Spirulina products, focusing specifically in East Africa, to enable possible food-based strategic interventions aimed at improving human nutrition in the region. A field survey was conducted to identify local Spirulina producers in East Africa and obtain samples. A total of 54 mice were used in evaluating the nutrient bioavailability and safety *in vivo*. A randomized control trial research design was used to select and allocate mice into treatment groups. Randomly grouped mice were separately fed three diets: control (basal), test (15% Spirulina powder blended with 85% basal diet) and standard (basal diet supplemented with standard nutritional supplements). The test and standard diets had equivalent nutrient content to enable direct comparison of results. Nutrients content analyses showed that a 100 g analyzed Spirulina samples contains 70 g protein, 82 mg iron, 84.5 mg zinc, 1302 mg calcium, 628 mg phosphorous, 27 μg vitamin A, 246.8 μg vitamin B_9, 3.99μg vitamin B_{12}, 1.92 mg QE/g total flavonoid and 2.99 mg GAE/g total phenolic. The nutrients bioavailability from the control diet was statistically lower than those of test and standard diets, whose results were comparable. After the feeding experiment, mice had no significant differences in their serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), cystatin C and troponin I concentrations, indicating that the studied concentrations of control, test and standard diets had no adverse effect on critical organs (liver, heart, or kidney) of the mice. These results indicate the safety of Spirulina and reinforce its importance in reducing undernutrition in humans.

DECLARATION

I, Feven Tezera Damessa do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Feven Tezera Damessa

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The above declaration is confirmed:

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Supervisor Name	**Signature**	**Date**

Dr. Sr. John-Mary Vianney

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Dr. Musa Chacha

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CERTIFICATION

The undersigned, hereby certify that have read the dissertation titled "*Nutritional and Functional Values of Microalgae (Spirulina) Naturally Found in East Africa*" and approved for submission in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology (NM-AIST).

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DEDICATION

This work is dedicated to my family.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg	Microgram
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CFSAN	Center for Food Safety and Applied Nutrition
CGIAR	Consultative Group on International Agricultural Research
CYSc	Cystain C
DHA	Docosahexaenoic Acid
DSI-EC	Dietary Supplements Information Expert Committee
DW	Dry Weight
EC	European Commission
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
g/dl	Gram per Deciliter
GIT	Gastrointestinal Tract
GLA	Gamma-Linolenic Acid
GRAS	Generally Recognized as Safe
HDLC	High Density Lipoprotein Cholestrol
HIV	Human Immunodeficiency Virus
IAASTD	International Assessment of Agricultural Knowledge, Science and Technology for Development
IFAD	International Fund for Agricultural Development
IFAS	Iron and Folic Acid Supplement
IPCC	Intergovernmental Panel on Climate Change
IU	International Unit
LDL	Low-Density Lipoprotein
LDLC	Low-Density Lipoprotein Cholesterol
mg/L	Milligram per Liter
mm	Millimeter
ng/mL	Nano gram per milliliter
nm	Nanometer

PEM	Protein-Energy Malnutrition
PTH	Parathyroid Hormone
RDA	Recommended Daily Allowance
SDG	Sustainable Development Goal
TC	Total Cholesterol
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
U/L	Units per Liter
UNICEF	United Nations International Children's Emergency Fund
UV	Ultra-Violate
WFP	World Food Program
WHO	World Health Organisation
β	Beta
ϒ	Gamma
ω	Omega

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

In July 2019, the United Nation's Food and Agriculture Organization (FAO) reported that, globally, more than 820 million people are hungry. The situation is most alarming in Africa as the region has the highest rates of hunger in the world (more than 250 million people) made worse by population increase, poverty and effects of climate change (FAO, International Fund for Agricultural Development [IFAD], United Nations International Children's Emergency Fund [UNICEF], World Food Program [WFP] & World Health Organization [WHO], 2019). Overpopulation leads to land resource scarcity, deforestation, overexploitation of natural resources and climate change (Mekuria, 2018). The Food and Agriculture Organization (FAO) projects that a 70% increase in agricultural production worldwide will be required in order to feed a global population expected to reach 9.1 billion by 2050 (FAO, 2019).

These days, resources are being extracted and consumed at an increasing rate worldwide, thus, seeking a sustainable and resource-efficient food source is required to sustain the growing populations. Fortunately, attitudes are increasingly changing and several regions around the world have had various forms of nutrition transition, leading to rapid changes in food systems. Already, FAO has proposed edible insects (grasshoppers, crickets and mealworms) as sustainable alternative to going meat-free and decrease demand on animal protein that often increase the strain on the environment. In this study, however, the study propose microalgae as a sustainable food solution for malnutrition and its various forms, focusing specifically in East Africa, to enable food-based strategic interventions aimed at improving human nutrition as well as counter the impacts of climate change on food production.

Microalgae consist of a diverse group of photosynthetic prokaryotic cyanobacteria and eukaryotic microorganisms that are present in fresh and marine water habitats (Brasil *et al.*, 2017; Hamed, 2016). They have enormous biodiversity including as many as 8 classes, 32 genera and 200 000 to 800 000 species (Odjadjare *et al.*, 2017; Usher *et al.*, 2014). The genus *Arthrospira*is continues to draw attention globally for food, nutraceuticals, pharmaceuticals and other application due to its suitable nutrient composition (Shao *et al.*, 2019). *Arthrospira platensis* and *Arthrospira maxima* are the most commercialized and abundantly available

species, however, the genus has more than 30 species. Spirulina is a commercial name for products from these two species (Jara *et al.*, 2018; Sánchez *et al.*, 2003).

Microalgae are rich in essential nutrients that are mostly inadequate in the African plant-based staple diets (Saranraj & Sivasakthi, 2014). They produce a great variety of secondary metabolites with high biological values (Jung *et al.*, 2019; Saranraj & Sivasakthi, 2014). Microalgae is naturally packed with high-quality protein, vitamins, minerals and other health-promoting bioactive compounds (e.g. phenolic acids, flavonoids, etc.). For instance, the protein content of microalgae is relatively high (about 50 to 70% of dry matter) compared to 18% in beef (Neumann *et al.*, 2002). Microalgae is rich in β-carotene (about 180 mg per 100 g of dry matter) which is enough to meet the daily requirement of an adult (Mobin & Alam, 2017). A small quantity (100 g) of microalgae contains approximately 60-300 µg vitamin B_{12} (cobalamin), which is normally inadequate in most vegetarian diets (Edelmann *et al.*, 2019; Mohan *et al.*, 2014). Microalgae contain a high level of absorbable iron (70-100 mg/ 100 g) compared to plant-based diets (Choopani *et al.*, 2016; Gumbo & Nesamvuni, 2017; Kumari *et al.*, 2011; Mohan *et al.*, 2014; Sotiroudis & Sotiroudis, 2013). Moreover, 100 g microalgae can contain up to 1 g of Gamma-linolenic acid, makes it the best-known source of this essential fatty acid after human milk (Jara *et al.*, 2018; Martin *et al.*, 2005).

The nutritional quality of microalgae has been reported in some studies. For instance, 90% protein digestibility was reported by Gutiérrez-Salmeán *et al.* (2015) as compared to the reference protein (casein). Forty percent or more absorbability of iron has also been reported (Baroux *et al.*, 2001; Peng, 2004). Due to its high nutrient content and biological values, microalgae are regarded as a potential food candidate to improve nutrition in developing countries where malnutrition manifested in all its forms (FAO, 2019). Studies from some resource-poor African countries demonstrated the potential of microalgae in improving nutrition. In Chad, for example, the consumption of microalgae had significantly improved the nutritional status of the vulnerable population; due to the high consumption of microalgae which is abundantly found in Lake Chad (Piccolo, 2011). A study from Burkina Faso also showed that the consumption of food mixed with microalgae had significantly improved the nutritional status of undernourished children (Simpore *et al.*, 2006). Unlike Chad and Burkina Faso, the nutritional values of microalgae have not been fully tapped in Eastern Africa. Thus, harnessing the nutritional potential of microalgae is important to tackle the widely prevalent malnutrition problems in East Africa.

1.2 Statement of the problem

Despite the natural abundance of Spirulina in Eastern Africa (Habib *et al.*, 2008; Piccolo, 2011), its nutritional and functional values have not been well studied. So far, there is little scientific information regarding the nutrient and phytochemical contents of Spirulina from Eastern Africa. Further, to the best of my knowledge, the nutrient bioavailability and safety of Spirulina available in the region have not been well studied or reported. Thus, this study aimed at evaluating the nutritional and functional values, nutrient bioavailability and safety of Spirulina naturally found in East Africa.

1.3 Rationale of the study

Since the chemical composition of Spirulina may vary between strain, environment and cultivation conditions as well as geographical location (Braga *et al.*, 2018; Fadel & Kamil, 2012; Fatemeh & Mohsen, 2016; Gatamaneni & Lefsrud, 2018; Sujatha & Nagarajan, 2013; Toyub *et al.*, 2011; Wells *et al.*, 2017), it is, therefore, important to evaluate the nutritional and functional values of Spirulina from Eastern Africa. The information will help practitioners incorporate Spirulina in food-based nutrition interventions for combating malnutrition which continues to be a challenge in the region.

1.4 Research objectives

1.4.1 General objective

The main objective of this study was to evaluate the nutritional and functional values of Spirulina naturally found in East Africa.

1.4.2 Specific objectives

The specific objectives of the research were:

(i) To profile the nutrients and phytochemicals contents of locally found Spirulina.

(ii) To evaluate the bioavailability of nutrients from the locally available Spirulina using the *in vivo* animal model.

(iii) To evaluate the safety of locally available Spirulina through toxicity marker analysis.

1.5 Research questions

To carry out the research objectives, the research was guided by the following research questions:

(i) What are the nutritional and functional values of locally grown Spirulina biomass?

(ii) Are nutrients from the locally available Spirulina bioavailable?

(iii) Are the locally available Spirulina products safe for consumption?

1.6 Significance of the study

Various interventions have been used to reduce malnutrition, however, the problem still persists in East Africa. As the rest of the world experience economic growth and enhance the income status of their residents, the poor in East Africa could continue experiencing the effects of malnutrition and its various forms, which would increase their vulnerability to the never-ending health, economic and environmental shocks. Thus, there is an urgent need to ensure that the consumers in the region have access to the knowledge and resources needed to achieve optimum nutrition. This study will establish baseline nutritional quality and safety understanding of locally found Spirulina in diets in order to promote extensive production, consumer acceptance, industry utilization and demand of the microalgae in food systems in East Africa.

1.7 Delineation of the study

The study focused on nutrients which are mostly insufficient in the African plant based staple diets. Spirulina samples used in this study were obtained from local producers, however, Spirulina found in the natural environment (Lakes) might have different nutritional characteristic.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of microalgae

The use of microalgae as a food source and food supplement has a long history in human life (García *et al.*, 2017; Jung *et al.*, 2019; Wells *et al.*, 2017). It is not exactly known when it started to be used as a human food or food supplement. However, as reported by Bernal Diaz del Castillo in 1520, the harvest of Spirulina microalgae was started in Mexico, from the lake Texcoco; the native Mexicans used to dry and sale the harvested Spirulina to the local market for human consumption and they used to call it *Tecuitlalt*, it means excrements of stones. The topic of Spirulina was not mentioned again until 1940. In 1940, Pierre Dangeard, a French phycologist found out about a cake called "*dihe*" which was a puree of blue-green algae from the African Lake Chad and was consumed by the tribe of Kanembu who lived around the Lake (Sánchez *et al.*, 2003).

The importance of Spirulina in human nutrition is due to its overall nutritional quality. The nutritional analysis on Spirulina has started after it is confirmed that "*dihe"* is made from Spirulina by Jean Leonard, the botanist in 1964 (Sánchez *et al.*, 2003). During that time, Jean Leonard was requested to conduct a study of systematic and detailed growth requirements, physiology of Spirulina and the algae bloom in the sodium hydroxide production system by a Mexican company called Sosa-Texcoco Ltd (Habib *et al.*, 2008).

Spirulina protein gained popularity as an alternative food source during the first and second World Wars. In the post-war period, the United Nations Food and Agriculture Organization (FAO) reported the increased rate of hunger and malnutrition and introduced the concept of protein deficiency. The report claimed 25% of the world's population had a deficiency of protein intake in their diet. Thus, several research projects that are looking for alternative sources of proteins from chlorella, Spirulina, some bacteria and molds were launched during that time. In 1950, investigations on the chemical composition and industrial applications and experimental cultivations of microalgae started by the United States and Japan. However, studies were accelerated after the release of the book called "Algal Culture from Laboratory to Pilot Plant" by Buriew, 1953, the book triggered research works around the world. In 1967, Spirulina was established as a "wonderful food source" by the International Association of Applied Microbiology (Sanchez *et al.*, 2003). The nutritional and medicinal applications, as

well as the importance of Spirulina in water treatment applications, have been extensively studied since 1970. The Food and Agriculture Organization (FAO) in 2008 reported that the production of algae culture was greater than 68 000 tons, China and Chile were the major contributing countries; the production of Spirulina in China has started in 1990 and the country had more than 80 factories by 1997 (Habib *et al.*, 2008).

Spirulina has been cultivated for several decades for human consumption in many Asian countries, Australia, Europe and the USA (Kumari e*t al.*, 2011). In 1973 the first pilot Spirulina production started, this pilot production in a year produced about 150 tons of dry Spirulina biomass. Thereafter, the Mexican SosaTexcoco Ltd raise the capacity of production to 300 tons per year a natural pond of 12 hectares. The annual value of Spirulina production represented a third of the company's income from the manufacture of powdered soda from the lake deposits. The population of living algae Spirulina still is available in Lake Texcoco even though production of Spirulina by Sosa-Texcoco ceased in 1995 (Habib *et al.*, 2008). Today, Spirulina is produced in more than 30 countries worldwide including Australia, Bangladesh, Côte d'Ivoire, Ecuador, France, Chad, Brazil, Philippines, Thailand, Togo, Mexico, Benin, Myanmar, Portugal, United States of America, Burkina Faso, China, Israel, Madagascar, Costa Rica, Italy, Chile, Spain, Cuba, Martinique, Vietnam, Japan, Peru and India (Habib *et al.*, 2008).

Despite the long-term utilization of Spirulina as a food source in different areas of the world, its commercial application and large-scale production are still recent. Because of the recent new companies starting each year, the Spirulina business sector is very dynamic. In Europe, mainly in France, there are different sizes of more than 150 companies mainly producing dietary supplements and nutraceutical products from Spirulina present (Caporgno & Mathys, 2018; Khan *et al.*, 2018).

A naturally rich and most complete source of organic nutrition Spirulina has gained interest among the scientific community because of its high content of polyphenols, phytosterols, polyketides, polysaccharides, carotenoids, halogenated compounds, mycosporine-like amino acids, lectins, protein, polyunsaturated fatty acids, minerals, vitamins and other various bioactive compounds (Choopani *et al.*, 2016; Gumbo & Nesamvuni, 2017; Kumari *et al.*, 2011; Mohan *et al.*, 2014).

Because of its health benefits, Spirulina has been named by the World Health Organization (WHO) as one of the greatest superfoods on earth (Chacón-Lee & González-Mariño, 2010). Globally, there are many ongoing investigations on the utilization of Spirulina biomass and its extraction for human nutrition. As Pulz and Gross (2004) pointed out that, to use the right Spirulina with the relevant properties regarding the products is important in the industries to achieve a successful Spirulina biotechnology, the use of such knowledge is important to create a wider area of influence of Spirulina in the food industry.

2.2 Nutritional and functional values of microalgae

Spirulina is one of the most promising high-quality proteins. It contains a high (60-70% of its dry weight) concentration of protein. The protein from Spirulina is of high quality containing all of the essential amino acids such as isoleucine (6.8%), leucine (10.9%) and valine (7.5%) than the standard protein sources such as milk, meat, or eggs (Falquet, 2006; Khan *et al.*, 2005).

Protein is the building block of the human structural components of cells and tissues; also many hormones and enzymes are made from protein. During the period of rapid growth such as childhood, adolescence, pregnancy and breastfeeding, adequate intake of protein is required due to the increased body demand as protein is essential cells and tissue growth (Gropper *et al.*, 2009). Due to its high nutritional quality, the importance of Spirulina protein for undernourished people especially children with kwashiorkor is apprehended. Also, protein from Spirulina could be advantageous for people having intestinal malabsorption, for older people with difficulty in digesting complex protein and for people having diets restriction.

Only a tiny amount of vitamins and minerals is needed by the body, thus, they often called micronutrients, however, a disease can be caused if failed to get even that tiny amounts. Similar to protein, vitamins and minerals are vital for the formation of cells, tissues, hormones, genetic materials and chemicals for the nervous system thus, they are vital for the regulation of various physiological functions such as growth, physical and emotional balance and health as a whole (Gropper *et al.*, 2009).

Spirulina contains relatively high concentrations of vitamin A, B_9 (folic acid), B_6 (pyridoxine), vitamin B_1 (thiamine), vitamin E, B_3 (nicotinamide), B_2 (riboflavin) and B_{12} (cyanocobalamin) (Dixit, 2018). Spirulina is rich in β-carotene which is ten times higher than that of carrot (Mathur, 2018). A tablespoon Spirulina provides 23 000 IU (14 mg) of β-carotene. Even though a high dose of vitamin A can be toxic, β-carotene from Spirulina as well as vegetables does not

cause toxicity (Falquet, 2006). The vitamin B_{12} content of Spirulina is higher than that of beef liver, chlorella (microalgae) or other sea vegetables (Falquet, 2006). Spirulina is the sole source of vegetarian vitamin B_{12} because vitamin B_{12} is rare to find the vitamin from plant source foods (Balaji, 2018; Falquet, 2006; Sáncbez *et al.*, 2003; Shao *et al.*, 2019). Spirulina contains several pigments like chlorophyll a, echinenone, ß-cryptoxanthín, zeaxanthin, myxoxanthophyll and oscilloxanthine (Edelmann *et al.*, 2019; Tang & Suter, 2015).

Spirulina is the richest source of calcium, zinc, phosphorus (Capelli & Cysewski, 2010; Mohan *et al.*, 2014; Sáncbez *et al.*, 2003; Santhosh *et al.*, 2016). The geographical location where the Spirulina is grown and the content of mineral in the growing water determines the mineral concentration of Spirulina (Michael *et al.*, 2019). Globally among women, children and the older population, iron is the most commonly deficient mineral. For a healthy immune system and strong red blood cells, it is important to have sufficient dietary iron intake (Gardner & Kassebaum, 2020). Spirulina contains a higher concentration of iron than that of other dietary sources of iron; a tablespoon of Spirulina provides up to 10 mg of iron (Masuda & Chitundu, 2019; Selmi *et al.*, 2011). The blue pigment of Spirulina and the soluble complexes formed by phycocyanin and other minerals during digestion is theorized to be the reason for the high bioavailability of iron from Spirulina, thus, iron from Spirulina is twice absorbable as iron from vegetables and most meats (Dasgupta, 2016; Wells *et al.*, 2017).

Carbohydrates, fats and proteins are macronutrients that are daily required by the human body. Carbohydrates are one of the main energy sources of the body organs' (heart, brain, muscle, kidney and central nervous system) main source of energy (Gropper *et al.*, 2009). Spirulina contains about 15 to 20% carbohydrates represented essentially by branched polysaccharides made up mainly of glucose and is structurally similar to glycogen (Pugh *et al.*, 2001). Spirulina is rich in inulin, a polysaccharide with high molecular weight, inulin is a dietary fiber essential for gut health and has immune stimulatory activity, this water-soluble carbohydrate accounts for between 0.5-2 percent of a dry Spirulina (Pugh *et al.*, 2001).

The fat content in Spirulina (5%) is far lower than other food sources; about 10 grams (one tablespoon) of Spirulina has only 36 calories and no cholesterol. Whereas, 80 calories and 300 mg cholesterol is found in egg yet egg and Spirulina provides a similar amount of protein (Falquet, 2006; Moreira *et al.*, 2011). Thus, Spirulina is low cholesterol, low fat but high protein source food.

Fatty acids are important for the pathways of biological signaling. Dietary polyunsaturated lipids change to lipid peroxide in the body to serve as a precursor for signaling mediators. Some examples of biological signaling that fatty acids play a major role includes are metabolic modulation and pathways of neuron, the peroxidation of low-density lipid and formation of eicosanoids (Innis, 2007). Eicosanoid signaling molecules responsible for immune responses, growth factors, chemotaxis and platelet aggregation (Uauy & Dangour, 2006).

Gamma-Linolenic Acid (GLA), the rare polyunsaturated fatty acid putative medicinal properties represent 49% of the total fatty acids in Spirulina, thus, Spirulina is considered as one of the best-known source of Gamma-linolenic acid following the human breast milk and vegetable oils such as blackcurrant seed, hemp oil and borage (Habib *et al.*, 2008). It is reported that 10 g of Spirulina provides over 100 mg of ϒ-linolenic acid. Spirulina is also rich in unsaturated fatty acids such as linoleic and oleic acids and also saturated fatty acids (Gumbo & Nesamvuni, 2017). Phosphatidylglycerol, sulfoquinovosyl diacylglycerol and monogalactosyl-diacylglycerol are among the major (20-25% each) lipids in Spirulina (Choopani *et al.*, 2016).

2.2.1 Spirulina in the fight against malnutrition

The nutritional and functional efficacy of Spirulina against malnutrition from human and animal experiments has been demonstrated. East Africa is the place where malnutrition is manifested in all of its forms. The most recent evidence shows that Africa had 58.7 million children under-five years were stunted; a higher proportion (23.9 million) of the stunted children were from the Eastern Africa sub-region (WHO, 2018). Micronutrient deficiency is one form of malnutrition and is an important public health concern in developing countries (Rasolofoson *et al.*, 2018). Micronutrient deficiencies occur when intakes and bioavailability of nutrients are inadequate to endure good health and development (Bouis & Saltzman, 2017).

Spirulina due to its high content of essential nutrients, natural abundance and ease of cultivation could be possible commodity to tackle malnutrition among the vulnerable population (Chacón-Lee & González-Mariño, 2010; Saha & Murray, 2018; Wells *et al.*, 2017). The reason why Spirulina has been used traditionally by various cultures for centuries could be its efficiency in treating complications from malnutrition. For instance, the local population lived around Lake Chad have noticeably prevented undernutrition during famine for years despite their nutrient-deprived diet (Habib *et al.*, 2008).

The effectiveness of Spirulina to improve nutritional status and health conditions has been demonstrated: the study collected anthropometric data after supplementing malnourished children with 10 g Spirulina demonstrated how effective Spirulina improved the nutritional status of malnourished children in Zambia (Masuda *et al.*, 2014). Also, a prospective study carried out among undernourished children reported that the daily supplementation of 10 g Spirulina had significantly improved stunting and wasting rates among undernourished children (Matondo *et al.*, 2016).

A study that assessed the supplementation effect of Spirulina among malnourished under-five years children in Cameroon reported a positive impact of Spirulina to tackle malnutrition through improving biomarkers level in the blood and weight of undernourished children (Modestine *et al.*, 2015). A randomized control trial study conducted in India reported that the daily consumption of a small amount of Spirulina had significantly improved the intellectual performance and hematological profile of school girls; the study recommended a free Spirulina supplementation in schools, most especially in the underprivileged regions for the better school performance of children (Rajbir *et al.*, 2004).

Spirulina supplementation showed a significant effect on lipid profile in HIV–infected antiretroviral patients in Cameroon. A prospective, single-blind, randomized, multicenter study had participants of HIV antiretroviral patients involved in control and intervention groups who consumed local diet only and Spirulina supplement combined with the local diet, respectively. The study reported a significant decrease in total cholesterol, LDL-cholesterol and triglycerides in the group of patients who consumed Spirulina. A change in the atherogenic index defined by the ratio TC/HDL-C substitutable by LDL-C/HDL-C and the TC/HDL decreased significantly in the patients taking Spirulina. The study concluded nutritional supplementation with Spirulina combined with a quantitatively and qualitatively balanced diet for at least six months can retard an exposition to lipid abnormalities in HIV-infected patients (Ngo-Matip *et al.*, 2014).

In a randomized control study to compare the effect of Spirulina versus soybean as food supplements on insulin-resistant HIV-infected patients, HIV-infected participants received 19 g of supplement (Spirulina or soybean) daily for 8 weeks. The study reported insulin sensitivity in HIV patients improved more when Spirulina rather than soybean was used as a nutritional supplement (Marcel *et al.*, 2011). Also, a comparative study conducted in Burkina Faso on the nutritional recovery of HIV-positive and HIV-negative malnourished children evaluated the

effectiveness of Spirulina as a treatment for child malnutrition and its positive impact on the nutritional rehabilitation of HIV-infected children. The study reported Spirulina supplementation showed clinical improvement including weight increase, improvement of hematological parameters and a decrease in the HIV viral load among HIV-infected undernourished children (Simpore *et al.*, 2005).

In a blind randomized cohort study, a daily supplement of 1.5 g Spirulina among pregnant women showed significantly high hemoglobin increment than pregnant women who were supplemented with a conventional iron (ferrous sulfate) 90 mg and folic acid supplementation 1 mg/day supplementation (Niang *et al.*, 2017).

A study carried out to investigate the possible protective role of Spirulina in modulating the hepatotoxicity induced by aluminum chloride in rats reported that co-administration of Spirulina with $AlCl_3$ alleviated the hepatotoxic effect of $AlCl_3$ through restoring oxidant-antioxidant balance. It significantly declined the level of lipid peroxidation and increased the activity of antioxidant enzymes in the liver. It diminished the oxidative effects of $AlCl_3$ on the RBCs membrane and maintained the hematological parameters near normal limits. Moreover, it decreased the elevated activities of liver enzymes (ALT and AST), triglycerides, total cholesterol, LDL-c and bilirubin concentrations and increased the levels of HDL-c, total proteins and albumin. Spirulina is a valuable hepatoprotective agent (Aita, 2014).

The study conducted to investigate the effect of Spirulina on serum antioxidant status and exercise-induced oxidative stress of athletes in comparison to a commercial antioxidant supplement had 3 groups, control group which did not receive any supplement, an experimental group I which received 3 g Spirulina and experimental group II which received 1 capsule of commercial antioxidant supplement. The results reported that supplementation of both Spirulina and commercial antioxidant supplement significantly increased serum antioxidant levels such as β-carotene, serum α-tocopherol and plasma ascorbic acid in both experimental groups. A significant decrease was shown in the level of malondialdehyde (a marker for oxidative stress) in two supplemented groups. The result confers supplementation with Spirulina and the commercial antioxidant supplement has no significant difference in improving serum antioxidant status and protection against exercise-induced oxidative stress (Kalpana, 2012).

In general, several studies reported the content of nutrients in Spirulina from different parts of the world. The existing evidence showed that these studies reported different nutrient content of Spirulina based on their geographical locations. The effect of environmental and growing conditions on the nutrient content of microalgae has been reported (Fatemeh & Mohsen, 2016; Wells *et al.*, 2017), therefore, the geographical location where the Spirulina samples collected or cultivated and the growing media used to culture or cultivate Spirulina could be the cause of variation in the reported content of nutrients.

Also, the available efficacy studies demonstrated the nutritional and functional qualities of Spirulina. Spirulina demonstrated a potential effect in improving nutritional status and immunity among malnourished and HIV-infected patients. However, almost all available efficacy studies are conducted in western Africa and India. This indicated that there is limited information from other parts of the world especially from East Africa where the prevalence of malnutrition is very high. Not only the efficacy studies but also studies quantifying nutrient content of Spirulina are scarce from East Africa. The existed information limitation perhaps restricted the utilization of Spirulina in the region. Therefore, the nutritional values of Spirulina and its efficacy need to be well studied.

2.3 Bioavailability of microalgae nutrients

The nutrition and functional values of Spirulina are not only influenced by the concentration of nutrients and bioactive compounds it contains, but the efficient digestion and absorption of these compounds would determine its nutritive value (Rein *et al.*, 2013).

The bioavailability of nutrients from Spirulina has been reported from various human and animal studies. After comparing the effect of Spirulina, calcium carbonate and high calcium milk on the serum calcium, magnesium, phosphate and alkaline phosphatase and femur bone characteristics, Ekantari *et al.* (2016) reported that the bioavailability of calcium from Spirulina is higher than calcium carbonate and high calcium milk in rats; the study reported better serum nutrient concentration and femur bone characteristics from rats who received Spirulina for 8 weeks than rats who received calcium carbonate or high calcium milk.

A study evaluated the effects of Spirulina on the storage and utilization of vitamins A and E by feeding diets containing 0, 2.7, 10.7, 18.7 and 26.7% Spirulina to rats for 6 weeks reported when the percentage of Spirulina is 10.7 or more the utilization of the die was decreased also

the plasma α-tocopherol levels were significantly reduced with increasing levels of Spirulina. Furthermore, liver retinoid levels of rats increased when Spirulina was added to the diet, indicating the conversion ability of the naturally occurring carotenoids in Spirulina to vitamin A. However, the plasma levels of retinol decreased when Spirulina was fed at 10.7% or more. Thus, the study demonstrated that Spirulina can significantly alter the storage and utilization of vitamins A and E at lower levels (Mitchell *et al.*, 1990). Madhubalaji *et al.* (2019) evaluated Spirulina as a source of vitamin B_{12} (cobalamin) through the modulation of vitamin B_{12} deficiency mediated physiological and biochemical changes in experimental animals.

An observational study which compared the serum retinol and β-carotene levels of women who consumed the traditional Spirulina-containing diet called *Dihe´* and women who did not consume *Dihe´* reported that serum retinol level was significantly higher in the Spirulina group than the other group; the two groups of women had similar diet consumption except for *Dihe´* (Soudy *et al.*, 2018). Also, the study used fecal nutrient content and serum vitamin A analysis after the children are fed on vitamin A deleted diet and then supplemented with Spirulina powder containing 1200 μg β-carotene and the standard vitamin A supplement reported that the absorption of total carotene from the ingested Spirulina ranged between 55.7 to 88.9% and absorption of β-carotene from Spirulina was between 63.5 and 86.8%. Also, serum retinol showed significant improvement in both Spirulina and vitamin A supplement groups. The study concluded that Spirulina has a similar effect same as the standard vitamin A supplement to treat vitamin A deficiency in children (Annapurna *et al.*, 1991).

A study was conducted to assess the effect of calcium on the bioavailability of iron from Spirulina replete anemic rats with an iron source from Spirulina, Spirulina and calcium carbonate, iron Sulphate, iron Sulphate and calcium carbonate, normal diet and normal diet and calcium carbonate. The findings from hematological measurements showed that the level of hemoglobin increased after feeding the diet with Spirulina, iron sulfate and a normal diet. The diet with added calcium carbonate slightly, but significantly, inhibited hemoglobin repletion after 21days in Spirulina but not in iron sulfate and normal diet. Further, the study demonstrated the hematological effect of Spirulina is similar to that of iron sulfate in rats. Thus, the study concluded that Spirulina is a good source of iron and it might be used as a multi supplement since it contains other nutrients too (Maznah, 2001). Also, the apparent biological value of microalgae protein has been reported to be 58 to 77%; bioavailability of fat from microalgae has been reported to be lower than that of soy oil (Neumann *et al.*, 2018).

Other human and animal studies also reported higher nutrient bioavailability and functional values from microalgae (Berg *et al.*, 1991; Gutiérrez-Salmeán *et al.*, 2015; Saharan & Jood, 2017). The available studies showed the nutritional potentials of different microalgae products on the nutritional status, blood level nutrient concentration, immune system, treatment of some diseases. However, almost all studies conducted to evaluate the nutrient bioavailability of microalgae are not from East Africa. Moreover, the most valuable studies on the topic are from old times which are conducted 10 or more years ago. Also, there is a huge limitation of information on the subject matter from East Africa.

2.3.1 Bioavailability assessment methods

The human digestive system is a complicated process and it involves various internal body organs and enzymes. Thus, the process could be affected by different factors at various stages. The structure of the digestive system includes organs that comprise the alimentary canal (gastrointestinal tract) as well as certain accessory organs. The mouth starts breaking up the food with the teeth and starts the digestion of sugars with enzymes. The tongue pushes the food to the back of the mouth where it is then swallowed and travels down the esophagus to the stomach. The esophagus does not digest the food, but it does the important job of pushing the food down into the stomach as well as keeping it from coming back up into the mouth. Once in the stomach, further digestion takes place (Doucet & Ball, 2016; Gropper *et al.*, 2009; Zewdie, 2018).

The stomach produces an acid that helps to kill bacteria and other germs that may get into food. The stomach makes an enzyme that starts the digestion of protein and releases a molecule that helps with the absorption of vitamin B_{12}. Once filled with food, the stomach grinds and churns the food to break it down into small particles. It then pushes the small particles of food into the first part of the small intestine, called the duodenum (Doucet & Ball, 2016; Gropper *et al.*, 2009; Zewdie, 2018).

The small intestine is where most of the digestion and absorption of our food takes place. In the small intestine, food is processed by different chemicals that are designed for specific components of the meal. Proteins, fats and sugars (carbohydrates) are digested by enzymes released by the pancreas. A tube from the pancreas joins the duodenum and all the enzymes travel together into the duodenum when food is present. A separate tube connects the liver and gallbladder to the duodenum. This tube allows bile, which is made by the liver and stored in

the gallbladder, to mix with food in the intestine. Bile is essential for complete fat digestion and the digestion of fat-soluble vitamins A, D, E and K. Once the sugars have been partially broken down by the enzymes of the pancreas, cells lining the small intestine use their enzymes to fully digest the sugars. Absorption takes place in the small intestine. Most of the digestion occurs in the first part of the small intestine while the absorption of broken-down nutrients, water, vitamins and minerals occurs in the rest of it. Eighty percent of the water ingested is absorbed in the small intestine. Once nutrients are absorbed by the intestine, they pass into the bloodstream and are carried to the liver (Doucet & Ball, 2016; Gropper *et al.*, 2009; Zewdie, 2018).

The liver has the job of processing all the nutrients, vitamins, drugs and other things ingested and absorb each day. It will turn protein, sugar and fat into energy which, with the help of pancreatic hormones like insulin, will feed the cells of our body. The liver also gets rid of the byproducts of drugs and the nutrients that are not needed in bile. Bile is the primary way the body gets rid of excess cholesterol and heavy metals such as copper. The large intestine, also called the colon, is not responsible for digestion. Instead, its purpose is to complete water and electrolyte (minerals found naturally in the body, such as potassium, calcium, sodium and magnesium) absorption begun by the small intestine. Those components of food that are not needed or cannot be absorbed are excreted from the colon in the stool. The color of the stool comes from the tiny amount of bile released from the liver each day that is not reabsorbed (Doucet & Ball, 2016; Gropper *et al.*, 2009; Zewdie, 2018).

The scientific community has been evaluating the bio-accessibility and bioavailability of nutrients from diets using different digestion models such as *in vivo* and *in vitro* models and it was able to accurately mimic the complex human digestive system (Courraud *et al.*, 2013). The fraction of bioactive compound which is released from the food matrix in the gastrointestinal tract and becomes available for absorption can be defined as bio-accessible; bio-accessibility includes the process of food transformation into material ready for absorption, absorption through intestinal epithelium cells and also the process of pre-systemic metabolism (Courraud *et al.*, 2013). The methods of simulating the gastric and small intestine digestive process in the *in vitro* digestion and Caco-2 cells uptake are commonly used methods to evaluate bio-accessibility (Courraud *et al.*, 2013).

Thus, bioavailability should be demonstrated as the analyzed compound is efficiently digested and absorbed and then exert an effect on health; however, due to ethical and practical

challenges to measuring bioactivity of a compound, the fraction of a given compound or its metabolite that reaches the systemic circulation usually defined as bioavailable (Holst & Williamson, 2008), without considering bioactivity. *In vivo* methods are mostly used to determine the bioavailability of compounds using animal or human subjects (Rein *et al.*, 2013). Tissue uptake and specific effect on the physiological response upon exposure to a substance used to measure bioavailability of a substance (Fernández-García *et al.*, 2012). Digestibility is defined as the fraction of nutrients and bioactive compounds that are transformed by the process of digestion into a material ready for assimilation and assimilation can be defined as the uptake of bio-accessible materials via the intestinal epithelium cells (Etcheverry *et al.*, 2012).

2.4 Safety of microalgae

Spirulina is widely used as a nutraceutical and pharmaceutical product worldwide due to its suitable content of nutritional and functional compounds (Shao *et al.*, 2019). However, there might be few safety concerns for consideration to protect public health. Exposures during cultivation, processing and packaging of Spirulina may cause contamination in the final product (Kay *et al.*, 2009). In agricultural areas, heavy metal contamination is common due to the usage of certain pesticides and fertilizers that contain toxic metals (Al-Dhabi, 2013). Moreover, it is noted that spirulina can bind heavy metal ions from the water and embed them in the cell vacuole (Biris-Dorhoi *et al.*, 2016). Pollutant and pesticide residues in the water may also be a problem (Kay *et al.*, 2009).

Few studies reported a lower concentration of heavy metals from commercial Spirulina products (Al-Dhabi, 2013; Al-Homaidan, 2006). Contamination of heavy metal from the natural environment including soil and water is very high (Jaishankar *et al.*, 2014). Since commercial or extensive Spirulina production usually takes place under controlled environmental conditions such as greenhouses, the available studies might not reflect the safety of Spirulina obtained in the natural environment such as lakes, rivers and other water bodies. Few animal studies assessed the physiological effects of microalgae consumption; these studies reported that microalgae do not exert a toxic effect on the internal body organs of consumers (Berg *et al.*, 1991; Neumann *et al.*, 2018; Janczyk *et al.*, 2005).

The Dietary Supplements Information Expert Committee (DSI-EC) reviewed and analyzed recent regulatory and pharmacopeial sources, human clinical trials and animal studies to assess the potential adverse effect of Spirulina. After reviewing this information, the DSI-EC

concluded that the available evidence does not indicate the adverse effect of Spirulina on human health and other public health concerns (Marles *et al.*, 2011). However, there is still a limitation of information from animal and human studies concerning the adverse interaction of Spirulina supplementation with pharmaceutical compounds or other dietary supplements. There are few reports on the side effects of ingestion of Spirulina, the few reported side effects are including headache, stomach ache, muscle pain, flushing of the face, sweating and concentration difficulties (Mazokopakis *et al.*, 2008).

However, few findings restrict the consumption of microalgae for a certain group of patients. For example, due to the immunomodulatory nature of Spirulina, the ingestion of Spirulina might affect disease severity in patients with autoimmune diseases, thus, patients with autoimmune diseases should avoid consumption of Spirulina (Kraigher *et al.*, 2008; Lee & Werth, 2004). Moreover, the neuroprotective effect of Spirulina through retarding or stopping motor neuron degeneration in a mouse model of Amyotrophic Lateral Sclerosis (ALS) has been reported by Garbuzova-Davis *et al.* (2010). However, as emphasized by the ALS Untangled Group (ALS Untangled Group, 2011), there is no enough scientific evidence that Spirulina is efficient for ALS. Besides, ALS Untangled Group suggested consumption of Spirulina could be toxic for patients with ALS. Thus, without enough information from human clinical trials and animal studies, Spirulina should not be consumed by patients with ALS (ALS Untangled Group, 2011).

In general, there are only a few human and animal studies conducted to evaluate the safety of microalgae as a human food or food supplement. Even though most available studies showed Spirulina has no toxic effect, yet there is still a dearth of clear information on the topic.

2.5 Microalgae and food insecurity

In Africa, undernutrition is a major public health problem causing morbidity and mortality among the vulnerable population (WHO, 2018). The Food and Agriculture Organization (FAO) report showed that the prevalence of undernourishment is rising in Africa; today, almost 256 million people are hungry in the region. The food and agriculture organization also mentioned climate extremes, population growth and economic slowdowns are the key drives of the rise in food insecurity (FAO, 2019).

Because microalgae are a rich source of essential nutrients (Kay *et al.*, 2009; Wells *et al.*, 2017) and they are responsible for more than half of the atmospheric oxygen production on earth (Piccolo, 2011) also they do have a positive impact on the environment (Kay *et al.*, 2009; Wells *et al.*, 2017); microalgae seem to be the future food that gives solution for the challenges Africa is facing.

2.5.1 Factors leading to food insecurity

In the 21st century, the available land for conventional agriculture is becoming reduced as population growth and demand increases, yet, food production needs to be doubled to supply the demand. By 2030, the world's population is predicted to increase by 50% and reach 9 billion; and by 2050, the African population alone is projected to be 2 billion. This situation leads to rapid urbanization which currently is increasing in most developing countries; 5 billion people globally are expected to live in urban areas by 2030 (United Nations, 2019).

Increased demand for food could be a direct effect of increased population size; similarly, altered dietary preference or demand for different food could be due to changes in incomes. For instance, economic factors such as income, price and lifestyle changes determine the growth rate of animal source food consumption (Thornton, 2010). Due to population growth, urbanization, shifts in income and changes in dietary preferences, the world's demand for animal source food is increasing and it is expected to increase more; the change is expected to be significant in developing countries (CGIAR Science Council, 2008). To meet the growing demand for food, it is expected to increase the amount of food production yet using the same land and water resource (Thornton, 2010). Due to population growth and expanding market, the traditional agricultural production used much land for cultivation. Moreover, the land now used for agricultural production is affected by soil degradation, salinization and pollution due to poor agricultural land management, thus, agricultural productivity and potential yield are low (Mekuria, 2018). Thus, the growing population size together with urbanization and unimproved agricultural production makes the idea of increasing agricultural productivity and food production difficult. Thus, improving agricultural techniques to less utilization of natural resources and producing large amounts of biomass is needed (Thornton, 2010).

The world's climate is continuing to change at an ever-increasing rate; the global average surface temperature is projected to be between 1.8 and 4.0°C from now to 2100 according to Model projections of the Fourth Assessment Report of the (Intergovernmental Panel on

Climate Change [IPCC], 2007), the range of temperature, of course, be depending largely on the models used and also the scale of fossil fuel burning between now and then. The impact of climate change is highly significant on agricultural productivity, with the changing temperature productivity of crops may alter (IPCC, 2007).

Crop yield is expected to fall by 10 to 20% in the tropics and subtropics by 2050 due to warming and drying. Moreover, the yield losses may be much more severe in some places (Dietz, 2020). It is also projected that there is a variability of climate change, however, there is uncertainty about the change. It is expected to have increased drought-affected area and also increased frequency of heavy precipitation; increased frequencies of drought, flooding and heat stress reduces crop and livestock productivities (IPCC, 2007).

The burden of climate change will be higher on the poor croppers and livestock keepers and the natural resources on which these farmers depend on; reduced rain-fed crops and forage, reduced water availability, increased livestock and crop diseases are consequences of climate change. Thus, major changes in the agricultural system are required (Dietz & Dietz, 2020). Yet there is natural resource scarcity, the future food production mainly depends on the increasing yield of crop and livestock productivities, especially from mixed systems (Mekuria, 2018). Since 1980, the rate of cereal yield increase has been slowing in developing countries despite the absolute yield of cereals increase. It might be possible that the future technological option can increase agricultural productivity without significant expansion on the lands for cultivation. It is known that improved agricultural technologies are the key drivers for growth in agricultural productivity (Hazell & Wood, 2008). Also, recently in the field of natural resource management, there are considerable developments. However, private sectors have less contribution to undertake natural resource management and public goods researches for the continuing globalization and privatization of agricultural science (Kesavan & Swaminathan, 2008).

According to the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD), neglecting investments in agricultural technology and science may lead to rapid degradation of agricultural supporting services and increased childhood malnutrition level. In general, modes investment levels and trading off improved crop productivity may help to achieve better outcomes in food security (McIntyre *et al.*, 2009).

Without improved agricultural technologies and a good natural resource management system, it will not be able to improve food production and meet the growing food demand. Decreased agricultural productivity leads to food shortages and malnutrition. Among the drivers of malnutrition, poverty-related factors, such as food insecurity and infectious diseases, persist as drought, floods and protracted humanitarian crises continue to mark the face of Africa (Carroll *et al.*, 2017). Recently, around one in seven people around the world are chronically hungry, lacking enough food to be healthy and lead active lives and the situation is more worse in Africa (FAO, IFAD, UNICEF, WFP and WHO, 2019).

2.5.2 The burden of undernutrition in Africa

World Health Organization, Africa regional office nutrition report showed that malnutrition is still persistent in the region. Among 47 countries of the region, 25 of them have high (>30%) or very high (>40%) rates of stunting. The report also showed that instead of decreasing to meet the global nutrition target of reducing the stunting rate in 2025, the rate of stunting is increasing in Africa (WHO, 2017). The recent evidence shows that Africa had 58.7 million stunted under 5 children in 2017, a higher proportion (23.9 million) of stunted children was from the Eastern Africa sub-region (WHO, 2018). The prevalence of wasting in 2017 was 13.8 million children, of whom 4 million were severely wasted, showing that undernutrition remains a serious public health problem in Africa. At the same time, the number of overweight under-fives rose from 6.6 million in 2000 to 9.7 million in 2017 (WHO, 2018).

Vitamin A deficiency is one of the major nutritional concerns in poor societies, especially in lower-income countries. Globally, night blindness affects 5.2 million preschool-age children and 9.8 million pregnant women, which corresponds to 0.9 and 7.8% of the population at risk of vitamin A deficiency, respectively. Low serum retinol concentration (<0.70 µmol/l) affects an estimated 190 million preschool-age children and 19.1 million pregnant women globally. This corresponds to 33.3% of the preschool-age population and 15.3% of pregnant women in populations at risk of vitamin A deficiency, globally (Sahile *et al.*, 2020).

According to the world health organization regional report, the highest prevalence (2%) of night blindness among preschool-age children is in Africa which is four-fold prevalent than in South-East Asia (0.5%). This means that the number of preschool-age children affected by night blindness is high in Africa (2.55 million preschool-age children) which accounts for half the prevalence of the world's affected children. Also, the prevalence of night blindness among

pregnant women is high in Africa (9.8%), yet South-East Asia has 9.9% night blindness affected pregnant women. Each region contains one-third (3 million) of the pregnant women affected globally. The report also showed that the prevalence of biochemical vitamin A deficiency as indicated by a serum retinol concentration <0.70 µmol/l among preschool children is higher in these two regions (Sahile *et al.*, 2020).

Stunting and wasting are the devastating results of poor maternal and early childhood nutrition, which can cause compromised physical growth, cognitive development and immune system. In the long term, malnutrition leads to developmental delay, increased risk of death, less participation in the community, earn less as an adult and low quality of life. Therefore, maternal nutrition should also be a concern for the development of a healthy and productive society (Ijarotimi, 2013).

The burden of micronutrient deficiency during pregnancy not only affects the mother, but it will also pass through generation to generation and leads to adverse pregnancy outcomes (Gernand *et al.*, 2016; Torheim *et al.*, 2018). Poor maternal nutrition during Preconception and antenatal has a critical impact on fetal growth and development and health and well-being of the mother and results in preterm delivery, low birth weight, infant mortality and morbidity, poor neurodevelopment of the infant and maternal mortality (Darnton-Hill & Mkparu, 2015; Ramakrishnan *et al.*, 2012). These could be addressed at every stage of a women's life cycle, especially during pregnancy (Darnton-Hill & Mkparu, 2015).

Nutrition of women before and during pregnancy plays an important role in optimizing pregnancy outcomes and determines the availability and supply of nutrients for the growing fetus (Ramakrishnan *et al.*, 2012). Inadequate intake and poor quality diet combined with an increased nutrient requirement imposed by pregnancy lead pregnant women to multiple micronutrient deficiencies and causes higher rates of low birth weight (Zeng *et al.*, 2008).

Micronutrient deficiencies during pregnancy are a consequence of poor quality diet (low in micronutrient content and high in antinutritional factors), parity (frequent pregnancy), short inter-pregnancy intervals (less than 6 months), increased physiological needs during pregnancy, inadequate health system (malaria and infection), socioeconomic status, traditional dietary practices during pregnancy (avoidance of foods rich in proteins and vitamins) (Bailey *et al.*, 2015; Darnton-Hill & Mkparu, 2015; Gernand *et al.*, 2016).

In Sub-Saharan Africa, environmental and economic conditions cause an extra burden on women's nutritional status. Women are involved in heavy workload which increases their nutritional requirement, contrary to this, the widespread poverty that they are facing disabled them from having enough and quality diet. Furthermore, frequent and short inter-pregnancy interval often leads African women to move from one pregnancy to the other without adequately replenish their body nutrient stores before the next pregnancy (Darnton-Hill & Mkparu, 2015; Lartey, 2018).

The high prevalence of (20%) low maternal BMI (<18.5 kg/m^2) reflects the prevalence of widespread malnutrition in African women which is the consequence of low pregnancy weight gain. The high prevalence (14%) of low birth weight among Sub-Saharan African infants is reflected by inadequate pregnancy weight gain (Lartey, 2018). The risk of mortality and morbidity is higher among low birth weight babies than normal birth weight babies. They are also at a higher risk of postnatal physical and cognitive growth retardation (Zeng *et al.*, 2008).

Due to pregnancy-related complications, more than 500 000 women die each year. From which 99% are in developing countries (Broek, 2018). Inadequate stores or intakes of micronutrients during pregnancy may predispose the mother to anemia, hypertension, preeclampsia/eclampsia, complications of labor and even mortality (Ha *et al.*, 2003; Jiang *et al.*, 2005; Lartey, 2018).

Iron deficiency anemia is a widespread problem among reproductive-age women, although per day an adult woman only needs about 1mg absorbable iron (Ha *et al.*, 2003; Seshadri, 2001). The problem is even worse in developing countries, affecting >50% of women during pregnancy (Broek, 2018). Observational studies showed a correlation between anemia in pregnancy and poor outcomes (Ha *et al.*, 2003; Ramakrishnan *et al.*, 2012). Iron supplementation during pregnancy has been recommended as standard care by national and international bodies to reduce poor pregnancy outcomes (Ha *et al.*, 2003).

Currently, there is scant reliable information regarding the magnitude of zinc deficiency in pregnant women however, some studies revealed that zinc deficiency is a public health problem in Africa. The study conducted in Ethiopia showed about 53.0% (95% CI: 49.3-56.7%) of pregnant women are zinc deficient (Gebremedhin *et al.*, 2011). A national study conducted in Cameron suggested a high risk of zinc deficiency throughout the country (Engle-Stone *et al.*, 2013).

Globally, among pregnant women, the prevalence of night blindness is 7.8%, affecting 9.8 million women. The prevalence of low serum retinol concentration (<0.70 µmol/l) is 15.3% affecting 19 million pregnant worldwide. The World Health Organisation regional estimate indicates that the highest percentage (9.8%) of night blindness among African pregnant women affecting 3% million women. The prevalence of low serum retinol concentration (<0.70 µmol/l) is 13.5% affecting 4 million pregnant women in Africa (WHO, 2009).

While malnutrition can manifest in multiple ways, the path to prevention is virtually identical. Adequate maternal and child nutrition together with an adequate health-care system allows developing a healthy and productive society. Various interventions including supplementation and bio-fortification of crops are used to reduce the burden of malnutrition. However, these interventions are costly and not sustainable. Thus, diversification of staple diet with locally available nutrient-rich foods could be the way forward.

2.5.3 Microalgae as a solution for the challenge

Microalgae are an abundantly available and nutrient-rich source of food (Habib *et al.*, 2008). Moreover, the production of microalgae requires less land area and inputs per unit production and there are no inedible by-products as in the case of other food crops. All these facts make microalgae a potential candidate to overcome the challenge of malnutrition especially in developing countries (Kay *et al.*, 2009; Saha & Murray, 2018).

Since microalgae are resistant to harsh growing conditions and climate change challenges such as arid areas, bodies of water that has high salt concentration and areas with water that has a high pH, which otherwise cannot be used to grow other cultivars, it competes less with other traditional crops (Kay *et al.*, 2009; Saha & Murray, 2018). Moreover, microalgae cultivation does not contribute to soil erosion, requires little or no pesticides or herbicides application and requires minimum energy for both production and processing. It does not cause environmental degradation. Besides, microalgae produce large amounts of biomass than any of the common cultivars per unit of time. Thus, Spirulina could be produced as a rapidly renewable food resource with minimal environmental hazard (Kay *et al.*, 2009; Wells *et al.*, 2017).

The high-quality nutritional values are not the only importance of microalgae, but producing it locally is more appealing than imported foodstuff. Moreover, product development using local resources based on local demands will also improve the acceptance of the product among the

target beneficiaries. Thus, local production of microalgae provides a solution not only to tackle undernutrition but also reduces the rate of unemployment. Besides, the production of microalgae allows diversification from the traditional crop production where land and other resources are scarce (Ahmad *et al.*, 2011; Caporgno & Mathys, 2018; García *et al.*, 2017; Khan *et al.*, 2018; Kovač *et al.*, 2013; Miranda *et al.*, 2017).

Therefore, due to its nutritional, economic and environmental importance, promoting local production of microalgae could help to achieve at least four sustainable development goals in developing countries such as no poverty Sustainable Development Goal (SDG 1), zero hunger (SDG 2), responsible consumption and production (SDG 12) and climate action (SDG 13).

2.6 Valuable applications from microalgae

Microalgae has a wide range of industrial applications. Its application for healthy food for humans, biofuel production and wastewater purification have been extensively reported. Microalgae can also be used for other industrial applications such as cosmeceuticals, fodder additives, food for aquaculture, bioremediation and dyes (Ahmad *et al.*, 2011; Caporgno & Mathys, 2018; García *et al.*, 2017; Khan *et al.*, 2018; Kovač *et al.*, 2013; Miranda *et al.*, 2017).

2.6.1 Microalgae as feed

A small amount of Spirulina biomass can serve to improve the growth and health status of livestock by increasing their protein intake and boost their immune system. Nevertheless, the applications in cattle feed are still limited, some studies done looked at the inclusion rates and effect on milk yield and quality and reported microalgae has led to a higher milk yield increase in cows due to its chemical composition, which influences both the biological activity of the ruminal flora and physiological status of the animal (Habib *et al.*, 2008; Kovač *et al*., 2013).

2.6.2 Microalgae as bio-fertilizer

Bio-fertilizer is another important of Spirulina. Since microalgae have increased water binding and nitrogen-fixing capacity and have high nutrient composition, it has been used as a bio-fertilizer to replace inorganic fertilizers and renew depleted soil structures. Spirulina can replace the usage of 25 to 30 kg of chemical nitrogen fertilizer per acre to cut environmental damages from the chemical (Habib *et al.*, 2008).

Since synthetic fertilizer usage is attributed to increased soil erosion and degrading the local ecosystem, the use of organic fertilizer which is cost-effective and eco-friendly has become the best option. The study used Spirulina along with cow dung manure reported the use of marine microalgal treatment exhibited increased growth performance at the early stage of growth, improved yield characteristics and increased seed germination (Dineshkumar *et al.*, 2019).

2.6.3 Microalgae as biofuel

Even though fossil fuel is among the commonly used energy sources, it has a significant contribution to global warming by increasing carbon dioxide and greenhouse emissions. Therefore, recently, replacing fossil fuel energy with biodiesel energy has received much attention worldwide. Considering the current climate change challenges and food insecurity problems, among the current feedstocks of biodiesel such as food crops, non-food crops and microalgae, it seems using microalgae (Spirulina) is the only sustainable source of biodiesel in the future. Spirulina along with other microalgae species is responsible for the production of more than half of atmospheric oxygen on earth while consuming vast amounts of the greenhouse gas carbon dioxide. Also, they compete less for agricultural land with other food crops, thus, they have considered as one of the most promising sustainable energy sources for biodiesel production in terms of food security and environmental protection (Ahmad *et al.*, 2011).

Since Spirulina is capable of synthesizing and accumulating significant quantities of lipids, it is regarded as a sustainable source of biodiesel. Research reports show that microalgae are 20 to 40 times more productive than other oil crops in terms of biofuel production. Therefore, microalgae are the major source of renewable energy production (Santhosh *et al.*, 2016).

2.6.4 Microalgae for wastewater treatment

Nowadays the focus of wastewater treatment has shifted to the use of microalgae (Spirulina) culture as a modern solution of wastewater tertiary treatment. Since water is the most necessary resource for the existence of life on earth, keeping it to higher quality is crucial. Technologies in wastewater treatment have been constantly evolving. These technologies are oriented to the removal of nutrients, dyes and antibiotics, radioactive substances, chemical and biochemical oxygen demand, heavy metals and microorganisms from wastewater (Biris-Dorhoi *et al.*, 2016; Miranda *et al.*, 2017).

The concept of using microalgae (Spirulina) for wastewater treatment is that Spirulina uses nutrients derived from the aquatic environment, while wastewater has an enormous amount of those nutrients that Spirulina can use for its development. Wastewater has different forms of nitrogen and phosphorus, of which if they are discharged improperly, can be the main source of pollution that can contribute to the eutrophication of surface water bodies and thus, threaten the balance of the aquatic ecosystem and safety of drinking water. Meanwhile, microalgae (Spirulina) have demonstrated an excellent capacity as a nutrients stripper, as they need those nutrients for their development (Biris-Dorhoi *et al.*, 2016; Miranda *et al.*, 2017). Different species of microalgae have been used and they have shown different percentages of nutrient removal capacity, however, studies proved that Spirulina can reduce the nitrogen content of wastewater up to 84% (Biris-Dorhoi *et al.*, 2016).

Beside their nutritional and functional values, microalgae have other enormous importance to improve human life economically, environmentally and also health-wise. Therefore, countries with low economic status, agricultural land scarcity, food shortage, increased rate of malnutrition, increased unemployment and increased rate of climate challenge could use extensive microalgae production as a development and malnutrition reduction strategy.

2.7 Microalgae cultivation

The production technology for Spirulina is rather simple and requires little upfront investment if climatic criteria (temperatures, light intensity), availability of agricultural fertilizers and bicarbonate, access to water and competence of local staff are ensured (Habib *et al.*, 2008; Piccolo, 2011). Unlined ditches or concrete ponds can be used for the cultivation of Spirulina. To ensure the turbidity of the water, simple devices that are driven by wind energy or hand can be used. Simple cloth filtration also is used to harvest mature Spirulina biomass. A fresh Spirulina can be mixed with the diet after washing it with freshwater or it can be preserved by immediate drying with no significant loss in quality or nutritional value (Habib *et al.*, 2008; Piccolo, 2011).

Growing Spirulina outdoor is quite different from growing it in the laboratory. A shift from steady-state standardized conditions in laboratory-grown cultures to outdoors where growth conditions differ constantly leads to the problem of contamination which otherwise is controlled in laboratory growth. Appropriate site choice for outdoor Spirulina production is crucial to have a reasonable yield of production. Since Spirulina grows better in high-

temperature conditions, the annual temperature regime and precipitation condition of the growing area should be checked for better production. The availability of unpolluted freshwater and the condition of air pollution is crucial to select a production site for Spirulina. The choice of container or pond is another factor that can affect productivity. Since microalgae are photosynthetic organisms, the availability of sunlight, which mainly depends on the design of the pond and the size of the pond should be carefully considered during the construction of the pond. Sufficient carbon dioxide supply to the pond, pH level and nutrients are other important factors for better outdoor microalgae production (Fatemeh & Mohsen, 2016; Gatamaneni & Lefsrud, 2018; Grobbelaar, 2009; Ren, 2014).

Spirulina can live and rapidly adapt to a complex natural habitat with extreme conditions like variable temperature, UV-irradiation, salinity and nutrients. There is better production of Spirulina at a higher pH (9-11) and high bicarbonate concentrations. Paddle-wheel-driven raceway ponds are usually used to culture Spirulina. Depending on the size of the pond flow velocity and optimal light absorption by the algal culture, the water depth in the pond varies from 300 to 500 mm. The productivity of Spirulina is highly influenced by the temperature; Spirulina grows better at the temperature between 35 and 37 °C. Due to the filamentous nature of Spirulina, harvesting is relatively easy. An inclined gravity screen or a vibrating screen filter could be enough for the harvest (Richmond, 2004).

Since Spirulina growth requires high temperature, lakes in central Africa such as Lake Chad and Lake Rombou also lakes along the Great Rift Valley in East Africa such as Nakuru and Elementeita in Kenya and Ethiopia's Lakes Aranguadi and Kilotes are the potential sites for the production of Spirulina (Habib *et al.*, 2008; Piccolo, 2011).

Therefore, considering the advantage of a favorable natural environment in East Africa, promoting large scale Spirulina production in the region could be used as a development strategy and an alternative approach to reduce hunger, poverty, malnutrition, disease, overexploitation of natural resources and also climate change challenges in the region.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Spirulina samples used in this study were obtained from local producers which are located at the shores of Lake Victoria, the side of Kenya (Kisumu City). These producers were selected because Spirulina production in East Africa is mainly concentrated in Kenya, in particular, Western Kenya along the shores of Lake Victoria (Piccolo, 2011). The first-ever Spirulina production in East Africa was established in Western Kenya by the intergovernmental organization called IIMSAM (intergovernmental institution for the use of microalgae Spirulina against malnutrition) registered under the United Nations Economic and social council. The organization gives training on how to cultivate Spirulina and provides the isolated pure Spirulina mother culture for local farmers to start their production (Piccolo, 2011).

3.2 Study design and sample size

A field survey was conducted to identify local Spirulina producers in East Africa and obtain samples. Locally prepared Spirulina products were only available in Kenya. Whereas, a randomized control trial study design was used for the bioavailability and safety of the nutrients evaluation experiments. The required number of animals for the experiment was calculated by the formula suggested by Dell *et al.* (2002).

$$\boldsymbol{n = 1 + 2C(\frac{s}{d})2}$$

Where, *s* is the standard deviation from the previous study, *d* is the difference to be detected and C is a constant dependent on the value of α (significance level) and power (1-β) selected. For α = 0.05 and 1-β = 0.8, C is 7.8 and 2C would be 15.6. For standard deviation (*s*) = 0.6 from the previous study (Madhubalaji *et al.*, 2019) and *d* = 0.57, the number of mice required for the experiment was 54.

3.3 Experimental animals

A total of 54 mice aged 5-8 weeks, weighing 21-38 g were used in the study. Mice were randomly selected and grouped into control (n=18), test (n=18) and standard (n=18) treatment groups. All the 54 cages were numbered and mice were caged individually at room temperature

with 12 h of light and dark cycle. All mice had *ad libitum* access to feed and water. The mice were habituated to the housing condition as well as the experimental intervention (feeding and handling) for 3 days before starting the experiment.

3.4 Feeding and treatment

Control group mice received a plain basal diet (a commercial layers' feed composed of maize, wheat bran, fishmeal, groundnut cake) once a day for four weeks. Test group mice received Spirulina powder (15%) blended with basal diet (85%). Standard group mice received a basal diet supplemented with casein protein, calcium carbonate, iron sulfate, zinc sulfate, phosphate, retinol, folic acid and cyanocobalamin mixture (accounts about 13% of the total diet) to receive nutrient levels equivalent to the test diet. In this experiment, the standard group was used to compare the bioavailability of nutrients from Spirulina with the bioavailability of nutrients from the conventional dietary supplements. Daily feed intake was recorded and the mice were weighed before and after the feeding experiment.

3.5 Feces, blood and liver samples collection

Apparent nutrient absorption and bioavailability of nutrients were evaluated after the mice had been fed on the diets for the experimental period. The apparent nutrient absorption of experimental diets was calculated using the following formula:

$$\text{Nutrient absorption (\%)} = \frac{\text{Total nutrient intake} - \text{Nutrient excreted in feces}}{\text{Total nutrient intake}} \text{ x } 100$$

Three consecutive days of feces sample collections were made to get enough samples after the mice had been fed the experimental diets for 27days (Safwat *et al.*, 2015). At the end of the experiment, mice were anesthetized and dissected and the liver was excised. The collected feces and liver samples were dried overnight in a vacuum oven at 60°C and powdered for further analyses. The concentrations of vitamin, mineral and protein from the samples were measured using the standard methods proposed by Sami *et al.* (2014), Al-Homaidan (2006) and Mæhre *et al.* (2018), respectively.

Before dissecting the mice, blood was collected by cardiac puncture using a 23G needle and serum separator tubes (SST). The collected blood was allowed to clot in an upright position for 60 minutes at room temperature and centrifuged at 2500 rpm for 15 minutes. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), cystatin C and troponin I were

analyzed by using an enzyme-linked immunosorbent assay (ELISA) (Elabscience, Houston, TX) kit as per manufacturer's instructions at AFYAMAX policlinic laboratory.

All the procedures (the feeding and handling of experimental mice) used to evaluate the nutrient bioavailability and safety of Spirulina were adopted from previously published related studies (Neumann *et al.*, 2018; Janczyk *et al.*, 2005; Kapoor & Mehta, 1993; Mitchell *et al.*, 1990). The experiment was conducted at Arusha Technical College (ATC).

3.6 Ethics approval

The use of animals in the study was approved by Kibong'oto Infectious Diseases Hospital-Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) Health Research Ethics Committee-KNCHREC (approval number: KNCHREC00026).

3.7 Nutrient content analysis

The content of nutrients in the analysed samples was measured in three replications and average values were taken.

3.7.1 Vitamin analysis

Analysis of vitamins A and B was performed as per the method proposed by Sami *et al.* (2014). Briefly, for vitamin A (β-carotene) estimation, 1 g pyrogallic acid, 70 mL ethanol and 30 mL (50%) KOH were added to 10 g powdered samples, stirred and refluxed for 40 minutes using a water bath at 50°C. Extracts were obtained using different ether concentrations (50, 30 and 20 mL). The extracts were neutralized by double-distilled water, then dehydrated using anhydrous sodium sulfate, further concentrated to 5 mL in a water bath (50°C) and then diluted to 10 mL by using methanol. The extracts of samples prepared were filtered through 0.46 μm membrane and HPLC analysis performed on Cyberlab LC-100 HPLC system (Cyberlab, India). For vitamin A, the XDB-C18 column was used (5 μm, 4. 6 × 150 mm) with methanol solvent and UV detection was recorded at 325 nm. Vitamin separation was based on isocratic elution and the flow rate of the solvent was maintained at 1 mL/minute. For β-carotene, the TC-C18 column was used (5 μm, 4.6 × 250 mm) with acetonitrile-methyl alcohol-ethyl acetate (88:10:2) solvent and UV absorbance was recorded at 453 nm. The stock solution of vitamin A was prepared by dissolving 20 mg retinol in 20 mL ethanol (1 mg/mL). The stock solution

of β-carotene was prepared by dissolving 20 mg β-carotene in 20 mL n-hexane (1 mg/mL). Thereafter, 20 μL of standard solution and extract was directly injected into the HPLC column and vitamin A and β-carotene estimated by comparing its retention times with that of the standard solutions.

For vitamin B analyses, a 25 mL H_2SO_4 (0.1 N) solution was added into 2 g powdered samples and incubated at 121°C for 30 minutes. The mixture was cooled, a 2.5 M sodium acetate were added to adjust the pH to 4.5, a 50 mg Takadiastase enzyme were added and then the mixture were stored at 35°C overnight. A Whatman No.4 filter was used to filter the mixture and the filtrate was diluted with 50 mL pure water and again filtered using a 0.45 mm micropore filter. Then the extract (20 μL) was injected into the HPLC system (Cyberlab LC-100 HPLC system, India). The stock solution of cyanocobalamin was prepared by dissolving 10 mg cyanocobalamin in 10 mL deionized water (1 mg/mL). Stock solution folic acid was prepared by dissolving 10 mg of folic acid in 10 mL of 0.1 mol L^{-1} NaOH (1 mg/mL). A reversed-phase (RP-HPLC) column (HC-C18; 250 × 4.6 mm) was used with isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH = 3.54) and UV absorbance was recorded at 270 nm.

3.7.2 Mineral and heavy metal analysis

Minerals and heavy metals were analyzed by using a method described by Al-Homaidan (2006) For that, 10 mL of nitric acid were added to 10 g of powdered samples. The mixture was heated for 10 minutes using a block digester (Avishkar Int., India). After cooling, 5 mL nitric acid were added, heated again for 30 minutes and the solution left as such for 10 minutes to cool. Then 2 mL distilled water, 3 mL hydrogen peroxide and 2 mL hydrochloric acid were added and the mixture heated again for another 10 minutes. Whatman filter paper 1 was used to filter the solution and diluted with distilled water to 100 mL. Standard stock solutions were prepared by dissolving pure metals into solvents at the concentration of 1000 mg/L, working standard solutions were prepared by diluting the stock solution into five concentrations using solvents. Thereafter, samples and standard solutions were injected into the atomic absorption spectrophotometer (Rayleigh WFX-210, China). Readings on atomic absorption spectrophotometer were taken at different wavelengths 248.3, 213.9, 422.7, 213.6, 193.7, 228.8, 253.7 and 283.3 nm for iron, zinc, calcium, phosphorus, arsenic, cadmium, mercury and lead, respectively.

3.7.3 Protein analysis

The protein content in the samples was estimated as per the Kjeldahl method described by Mæhre *et al.* (2018). Briefly, 1 g of powder sample was hydrolyzed with 25 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets on a heat block (Behr Labor-Technik Gmbh D-40599, Germany) at 370°C for 2 h. After cooling, the flask was put into the distillation apparatus, 250 mL distilled water were added followed by 100 mL sodium hydroxide and the solution was heated at 100°C for 10 minutes and the distilled solution was collected in a flask containing 25 mL boric acid (4%). Thereafter, titration of the solution with hydrochloric acid followed by the addition of 5 drops of Tashiro indicator was performed. The traditional conversation factor of 6.25 was used to convert the amount of total nitrogen into protein values for different samples.

3.7.4 Bioactive compounds analysis

Total flavonoid content was analyzed as per the aluminum chloride colorimetric method proposed by Chandra *et al.* (2014). For this, 75 mL (95% v/v) ethanol was used to extract a 10 g powder spirulina sample. The dried extract was prepared by evaporating the solvent at 40°C under reduced pressure. Thereafter, a 5 mL methanol were used to dissolve 30 mg dried extract and the mixture was sonicated at 40°C for 45 minutes and centrifuged for 10 minutes at 1000 revolutions per minute. Then 0.6 mL of 2% aluminum chloride were added to 0.6 mL extract and mixed and incubated at room temperature for 60 minutes. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol and the solution was diluted to different concentrations (5-200 μg/mL) using methanol. Thereafter, 0.6 mL of 2% aluminum chloride were mixed with 0.6 mL diluted standard quercetin solutions of different concentrations separately. Ultraviolet (UV) detection was measured at 420 nm using a spectrophotometer (Rayleign vis-723G, China). Total flavonoid content was calculated as mg quercetin equivalent (QE)/g from the calibration curve of quercetin.

Total phenolic content was analyzed as per the Folin and Ciocalteu reagent method proposed by Chandra *et al.* (2014). For this, 75 mL (95% v/v) ethanol were used to extract a 10 g powder spirulina sample. The dried extract was prepared by evaporating the solvent at 40°C under reduced pressure. Thereafter, a 5 mL methanol were used to dissolve 30 mg dried extract and the mixture were sonicated at 40°C for 45 minutes and centrifuged for 10 minutes at 1000 rpm. Then 0.2 mL Folin-Ciocalteu's phenol reagent (1:1) and 0.6 mL water werw mixed with 0.2

mL extract and after 5 minutes the solution was mixed with 1 mL saturated sodium carbonate solution (8% w/v in water) and made the volume up to 3 mL with distilled water and incubated in the dark for 30 minutes. A 20 mg gallic acid were dissolved in 100 mL methanol to prepare the stock gallic acid solution and methanol was used to dilute 5-200 μg/mL diluted standard solutions. The standard solution was also prepared by using the same method as the extract. UV detection was measured at 765 nm using a spectrophotometer (Rayleign vis-723G, China). The phenolic content was calculated as gallic acid equivalents GAE/g from the calibration curve of gallic acid.

3.7.5 Phytic acid analysis

Phytate was determined by using an anion-exchange method following a method described by Ma *et al.* (2005). For this, a mixture of a 100 mL 3.5% HCl with a 5 g Spirulina sample was shaken at room temperature for an hour and then the extract was centrifuged for 10 minutes at 3000 rpm and 5 mL supernatant were diluted into 25 mL distilled water. Thereafter, 10 mL of the diluted solution were injected into a glass column stoppered packed with anion exchange resin of 0.5 g (AG1 X4, 200–400 mesh, Sigma). Fifteen milliliter of 0.1 M NaCl were used to eliminate the interference of inorganic phosphorus and other compounds. Fifteen milliliter of 0.7 M NaCl were used to elute the phytic acid in the column. Three milliliter of this eluate were transferred into a glass tube and mixed with Wade solution and then vortexed and centrifuged at 3000 rpm for 10 minutes. The absorbance was measured against Wade solution as a blank at 500 nm using a spectrophotometer (Shimadzu 1601PC UV, Japan). The calibration curve was constructed using phytic acid standard (P-8810 Sigma Co.).

3.8 Data analysis

Statistical analysis was performed using the statistical software IBM SPSS (23). Descriptive statistics were performed and the results reported as mean $\pm$ SD. Mean differences between groups were evaluated using one-way ANOVA (between-group variation) at a significance level (p-value) less than 5%. Normal distribution was evaluated with the Kolmogorov-Smirnov test. Equality of variances was evaluated using Levene's test. Tukey's HSD post hoc test was used to identify the specific difference between group means.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Nutrient content of Spirulina

The results from the nutrient content analysis showed that Spirulina naturally found in East Africa contains a substantial concentration of essential nutrients and bioactive compounds which are vital for human nutrition (Table 1, 2 and 4).

Regarding the anti-nutritional factor, the results showed that the locally found Spirulina contains very little concentration of phytate, 1.86 mg/100 g. Table 3 summarizes the computed mineral to mineral and phytate to mineral molar ratios together with the critical values at which mineral absorption could be inhibited.

Table 1: Vitamin A, vitamin B_9, vitamin B_{12} and protein contents of Spirulina naturally found in East Africa

Nutrients	Mean (SD)
Vitamin A (μg/100 g)	27 (1.39)
Vitamin B_9 (μg/100 g)	246.8 (11.02)
Vitamin B_{12} (μg/100 g)	3.99 (0.73)
Protein (g/100 g)	70 (2.4)

Table 2: Iron, zinc, calcium, phosphorus and phytate concentrations in Spirulina naturally found in East Africa

Nutrients	Mean (SD)/mg/ 100 g
Iron	82 (2.09)
Zinc	84.5 (5.6)
Calcium	1302 (1.67)
Phosphorous	628 (44.2)
Phytate	1.86 (0.08)

Table 3: Phytate to iron, phytate to zinc, phytate to calcium, iron to zinc, zinc to iron, calcium to phosphorous molar ratios of locally found Spirulina

Sample	Phytate/Fe	Phytate/Zn	Phytate/Ca	Fe/Zn	Zn/Fe	Ca/P
Spirulina	0.001 9	0.002 1	0.000 086	1.14	0.88	1.6
[a] Critical level	>1	>15	>0.24	>2	>2	>2

[a] Me *et al.*, 2009; Nguyen *et al.*, 2012

Table 4: Total phenolic and total flavonoid concentrations in locally found Spirulina

Nutrients	Mean (SD)
Total phenolic (mg GAE/g)	2.99 (0.48)
Total flavonoid (mg QE/g)	1.92 (0.35)

4.1.2 Bioavailability of Spirulina nutrients

(i) Bodyweight of experimental mice

The bodyweight of control, test and standard group mice before the experiment was 28.4 $\pm$ 4.98 g, 26.6 $\pm$ 4.37 g and 29.6 $\pm$ 4.10 g, respectively. At the end of the feeding experiment, the weight of control, test and standard group mice was 37.3 $\pm$ 5.41 g, 36.3 $\pm$ 5.03 g and 38.3 $\pm$ 5.12 g, respectively.

The weight of the mice as measured at the start and end of the experiment (Fig. 1) did not vary significantly between the different dietary treatments group ($p > 0.05$). However, a paired sample t-test showed that mice had a significant weight change at the end of the experiment compared with the initial bodyweight they had ($p < 0.01$).

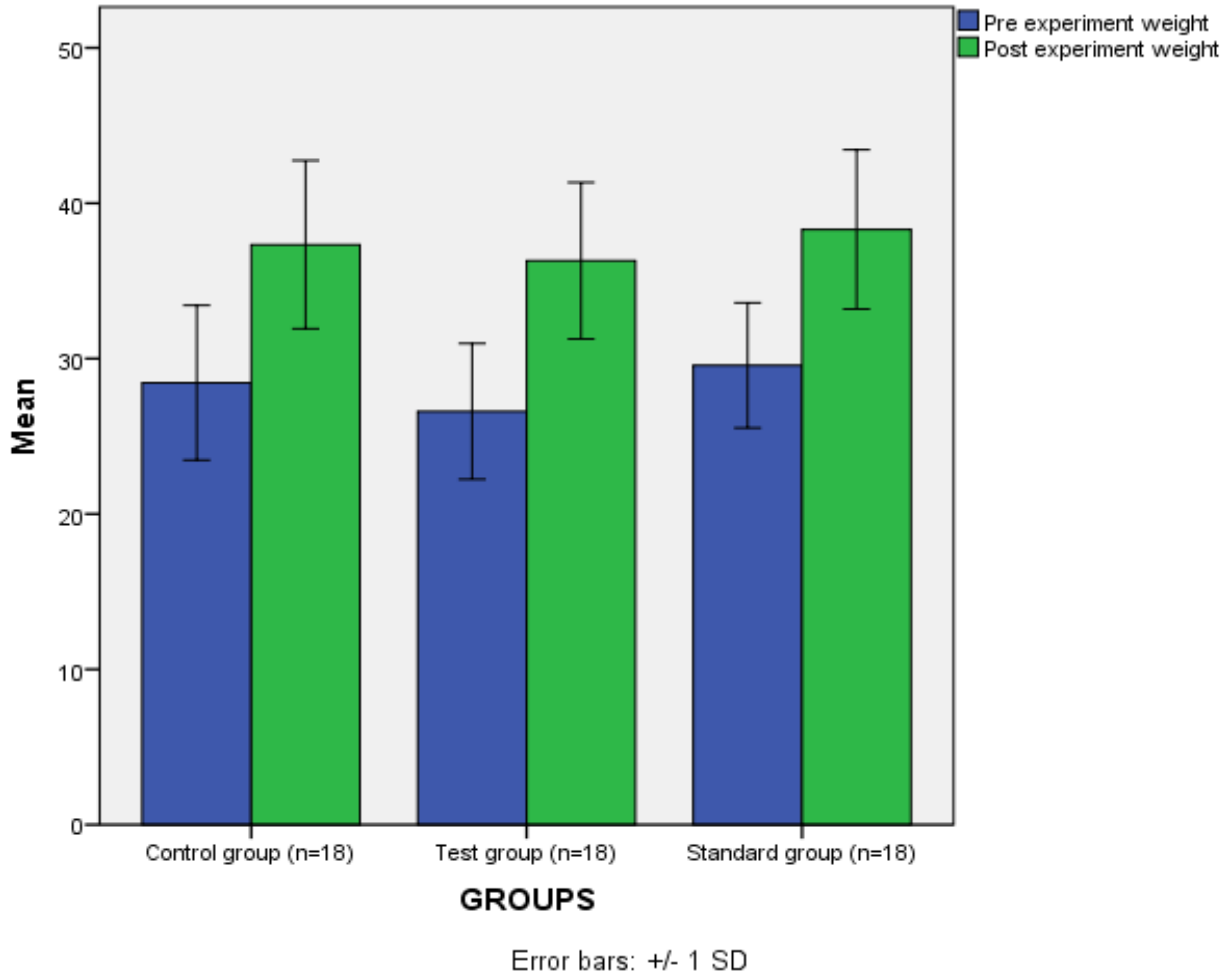

Figure 1: The weight of the mice was not statistically different (P > 0.05) between groups as measured before and after the experiment. However, mice had significant weight increments after the treatment (p < 0.01). The blue and green bars represent the mean weight of experimental group mice while the error bars represent the standard deviations

(ii) Feed intake

The daily feed intake of control, test and standard group mice (Fig. 2) was 4 $\pm$ 1.4 g, 4.3 $\pm$ 2.2 g and 3.3 $\pm$ 1.5 g, respectively, which was not significantly different (p > 0.05). Further, the total feed intake of control, test and standard group mice during the experiment (Fig. 3) was 99 $\pm$ 29 g, 104 $\pm$ 46 g and 83 $\pm$ 32 g, respectively, also not affected statistically (p > 0.05).

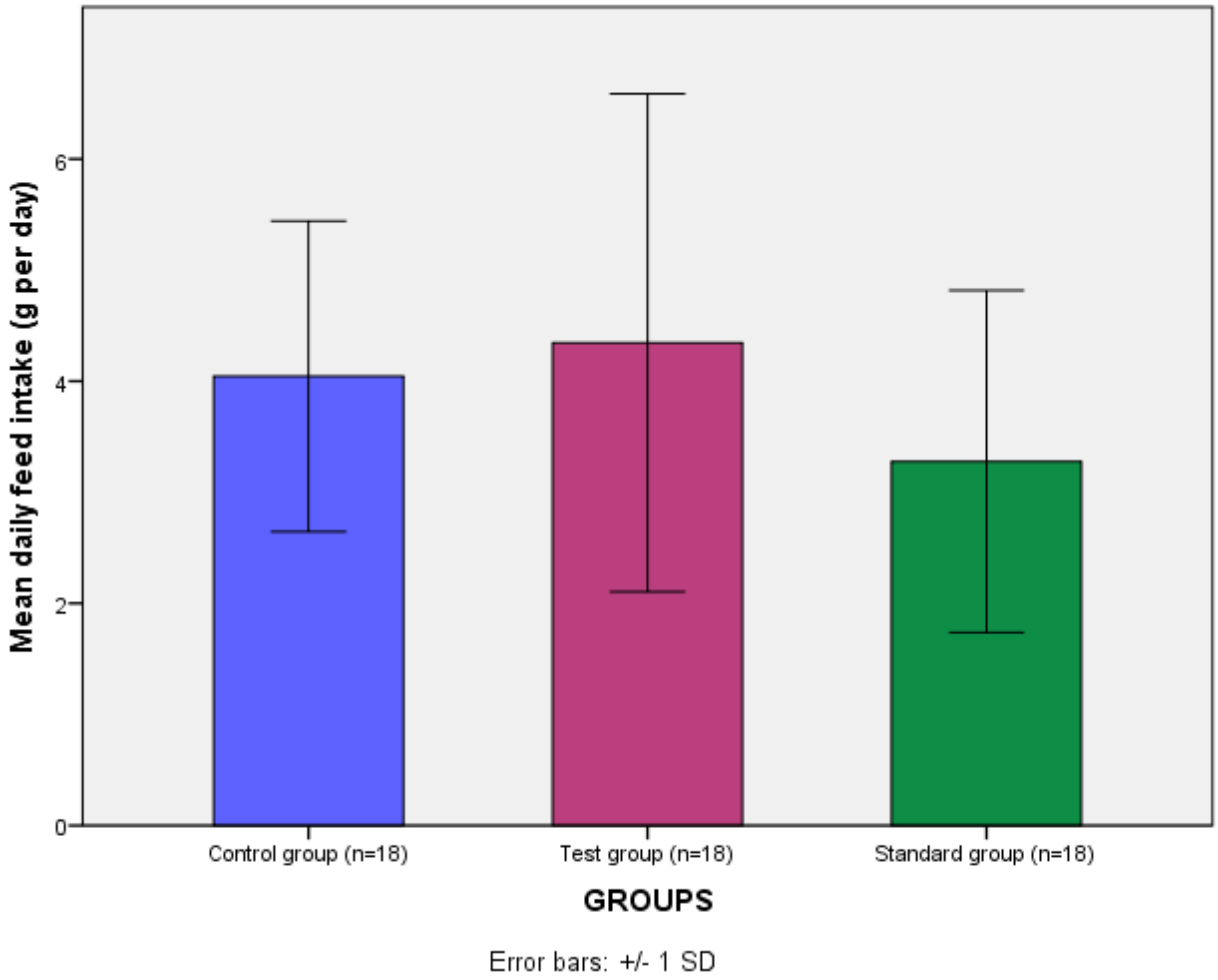

Figure 2: The daily consumption of feed was not statistically different between control, test and standard groups ($p > 0.05$). However, standard group mice had slightly lower intake (not statistically different) than the two other groups. Bars represent the mean daily feed intake of each group of mice while the error bars represent the standard deviation values

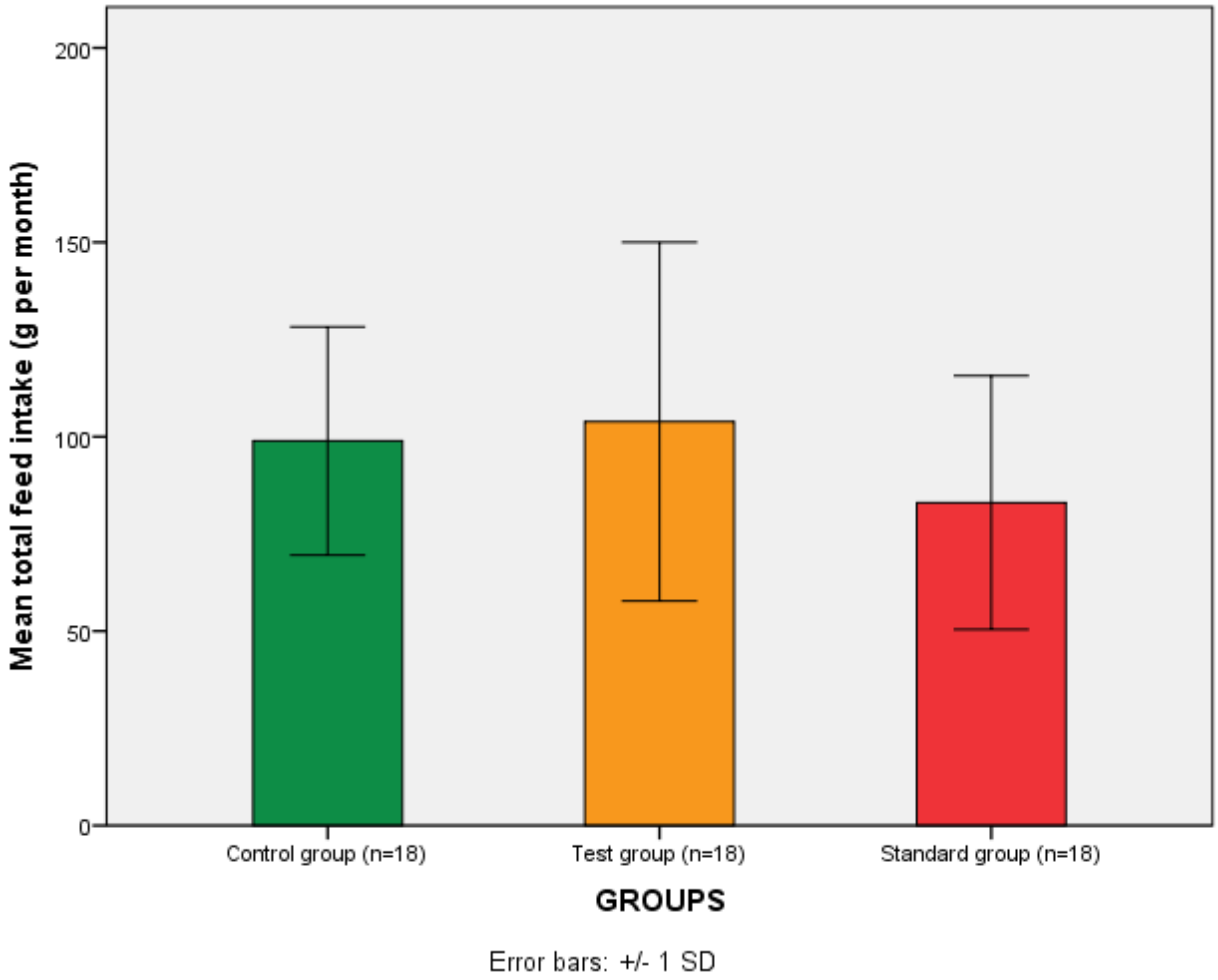

Figure 3: Consumption of feed during the four weeks feeding experiment was not statistically different between control, test and standard groups ($p > 0.05$). Bars represent the mean values of feed consumed by each group of mice during the four weeks experiment and the error bars represent the standard deviations

(iii) Nutrient content of experimental diets

The nutrient content of the control diet was significantly lower than the test and standard diets ($p < 0.01$). However, the nutrient contents of the test and standard diets were similar ($p > 0.05$) except for iron, zinc, phosphorous and vitamin B_9 concentrations (Table 5).

Table 5: Nutrient content of control, test and standard diets used in the feeding experiment

Nutrients	Units per 100 g		
	Control diet [a]	**Test diet [b]**	**Standard diet [c]**
Protein (g)	10 [b, c]	14.5 [a]	15 [a]
Calcium (mg)	199.4 [b, c]	364.8 [a]	364.5 [a]
Phosphorus (mg)	46.3 [b, c]	133.5 [a, c]	133 [a, b]
Iron (mg)	6.75 [b, c]	18 [a, c]	17 [a, b]
Zinc (mg)	0.9 [b, c]	13.4 [a, c]	12.3 [a, b]
Vitamin A (μg)	3.1 [b, c]	6.65 [a]	6.6 [a]
vitamin B_9 (μg)	11.1 [b, c]	231.8 [a, c]	229.5 [a, b]
vitamin B_{12} (μg)	0.2 [b, c]	0.77 [a]	0.8 [a]

Mean values with the letter 'a' mark a significant difference with the control diet (ANOVA, $p < 0.05$). Mean values with the letter 'b' mark a significant difference with the test diet (ANOVA, $p < 0.05$). Mean values with the letter 'c' mark a significant difference with a standard diet (ANOVA, $p < 0.05$)

(iv) Nutrient intake

The nutrient intake of mice during the four weeks feeding period (Table 6) was calculated based on the amount of feed ingested during the experiment (as presented in Fig. 3) and the nutrient content of experimental diets (as presented in Table 5).

The nutrient intake of control group mice was significantly lower ($p < 0.05$) than test group mice. Also, the nutrient intake of control group mice was significantly lower ($p < 0.05$) than standard group mice except for protein and calcium intakes. Further, the nutrient intake of test and standard group mice was almost similar ($p > 0.05$) except for zinc intake.

Table 6: Nutrient intake of mice from the control, test and standard diets during the experiment

Nutrients	Mean (SD)		
	Control [a]	Test [b]	Standard [c]
Protein (g)	9.9 (2.92) [b]	15 (6.7) [a]	12.4 (4.89)
Calcium (mg)	204.7 (62.9) [b]	379 (168.09) [a]	302.5 (118.8)
Phosphorus (mg)	45.8 (13.5) [b, c]	138.8 (61.5) [a]	110 (43.4) [a]
Iron (mg)	6.7 (1.97) [b, c]	18.7 (8.29) [a]	14 (5.54) [a]
Zinc (mg)	0.89 (0.26) [b, c]	13.9 (6.17) [a, c]	10 (4.01) [a, b]
Vitamin A (μg)	3.07 (0.9) [b, c]	6.9 (3.06) [a]	5.5 (2.15) [a]
Vitamin B_9 (μg)	11 (3.25) [b, c]	241 (106.8) [a]	190.5 (74.9) [a]
Vitamin B_{12} (μg)	0.19 (0.06) [b, c]	0.8 (0.35) [a]	0.7 (0.26) [a]

Mean values with the letter 'a' mark a significant difference with the control group (ANOVA, $p < 0.05$). Mean values with the letter 'b' mark a significant difference with the test group (ANOVA, $p < 0.05$). Mean values with the letter 'c' mark a significant difference with the standard group (ANOVA, $p < 0.05$)

(v) Feces samples nutrient content

Fecal nutrient content indicates how much of the ingested nutrient is absorbed in the body and how much is excreted. Table 7 presents the nutrient content of feces samples collected from control, test and standard group mice.

Statistical analysis showed that protein, calcium, phosphorous, iron, zinc, vitamin B_9 and B_{12} contents of feces samples collected from control, test and standard group mice was significantly different ($p < 0.05$). Feces vitamin A content was significantly different between control and test and also test and standard groups ($p < 0.05$) but not between control and standard groups.

Table 7: Nutrient content of feces samples collected from mice that consumed control, test and standard diets (unit/100 g)

Nutrients	Mean (SD)		
	Control [a]	Test [b]	Standard [c]
Protein (g)	7.3 (0.37) [b, c]	4.9 (0.54) [a, c]	4 (0.37) [a, b]
Calcium (mg)	173.4 (0.62) [b, c]	187 (0.63) [a, c]	156 (0.35) [a, b]
Phosphorus (mg)	39.4 (1.02) [b, c]	94 (0.85) [a, c]	72 (0.68) [a, b]
Iron (mg)	5.2 (1.21) [b, c]	10.5 (0.39) [a, c]	8.5 (0.59) [a, b]
Zinc (mg)	0.58 (0.06) [b, c]	8 (0.29) [a, c]	5.5 (0.60) [a, b]
Vitamin A (μg)	1.7 (0.10) [b]	3 (0.37) [a, c]	1.5 (0.46)[b]
Vitamin B_9 (μg)	7.9 (0.54) [b, c]	150 (0.71) [a, c]	115 (0.55) [a, b]
Vitamin B_{12} (μg)	0.15 (0.05) [b, c]	0.4 (0.08) [a, c]	0.35 (0.03) [a, b]

Mean values with the letter 'a' mark a significant difference with the control group (ANOVA, $p < 0.05$). Mean values with the letter 'b' mark a significant difference with the test group (ANOVA, $p < 0.05$). Mean values with the letter 'c' mark a significant difference with the standard group (ANOVA, $p < 0.05$).

(vi) Apparent nutrient absorption

The amounts of nutrients ingested during the experiment and the concentration of nutrients in the excreted feces samples were used to calculate the percentage of absorbed nutrients from the diets (Table 8).

Statistical analysis showed that nutrients absorption from the control diet was significantly lower ($p < 0.01$) than the test and standard diets except for zinc and vitamin B_9 absorptions. Further, the nutrients absorption from test and standard diets was similar ($p > 0.05$) except for vitamin A absorption.

Table 8: Apparent absorption of nutrients from the control, test and standard diets used in the feeding experiment (%)

Nutrients	Mean (SD)		
	Control diet [a]	Test diet [b]	Standard diet [c]
Protein	26 (8.1) [b, c]	67 (9.9) [a]	67.7 (10.7) [a]
Calcium	12 (4.4) [b, c]	50.6 (13.2) [a]	48 (12.8) [a]
Phosphorus	13.9 (4.6) [b, c]	32 (12.6) [a]	34.5 (14.3) [a]
Iron	22 (4.6) [b, c]	43.8 (13.9) [a]	39 (12.3) [a]
Zinc	34.8 (8.7)	42 (16.3)	45 (13.5)
Vitamin A	44.6 (7.6) [b, c]	56.5 (11.1) [a, c]	72.7 (5.2) [a, b]
Vitamin B_9	28 (9.9)	37.7 (19.3)	39.6 (9.6)
Vitamin B_{12}	21 (6.6) [b, c]	50 (13.1) [a]	50 (12.3) [a]

Mean values with the letter 'a' mark a significant difference with the control diet (ANOVA, $p < 0.05$). Mean values with the letter 'b' mark a significant difference with the test diet (ANOVA, $p < 0.05$). Mean values with the letter 'c' mark a significant difference with the standard diet (ANOVA, $p < 0.05$).

(vii) Liver nutrient content

The mineral and vitamin contents of liver samples collected from control, test and standard group mice were measured to evaluate how much of the ingested nutrients are absorbed by the body and carried to liver tissue as a nutrient bioavailability measuring parameter (Table 9).

Statistical analysis showed that the nutrient content of liver samples collected from control group mice was significantly lower ($p < 0.01$) than that of test and standard group mice. Similarly, liver nutrient content from test group mice was significantly higher ($p < 0.01$) than that of standard group mice except for iron, vitamin A and vitamin B_{12} contents.

Table 9: Nutrient content of liver samples collected from the mice consumed control, test and standard diets (unit/100 g)

Nutrients	Mean (SD)		
	Control [a]	Test [b]	Standard [c]
Calcium (mg)	22.5 (0.34) [b, c]	182 (1.24) [a, c]	148 (0.38) [a, b]
Phosphorus (mg)	6.4 (0.6) [b, c]	43 (0.74) [a, c]	38 (0.64) [a, b]
Iron (mg)	1.5 (0.07) [b, c]	8 (0.43) [a]	7.7 (0.53) [a]
Zinc (mg)	0.3 (0.08) [b, c]	5.8 (0.38) [a, c]	5 (0.39) [a, b]
Vitamin A (μg)	1.3 (0.54)[b, c]	2.4 (0.7) [a, c]	3.6 (0.85) [a, b]
Vitamin B_9 (μg)	2.9 (0.43) [b, c]	87.3 (1.3) [a, c]	73.8 (1.48) [a, b]
Vitamin B_{12} (μg)	0.03 (0.01) [b, c]	0.3 (0.05) [a]	0.31 (0.02) [a]

Mean values with the letter 'a' mark a significant difference with the control group (ANOVA, $p < 0.05$). Mean values with the letter 'b' mark a significant difference with the test group (ANOVA, $p < 0.05$). Mean values with the letter 'c' mark a significant difference with the standard group (ANOVA, $p < 0.05$).

4.1.3 Safety of Spirulina

(i) Heavy metal concentration

The safety issues regarding Spirulina consumption are mainly associated with its chemical composition. In this study, heavy metals concentration in Spirulina products was measured as a safety parameter. The results showed that the concentration of mercury, lead, cadmium and arsenic was 0.000 036 mg/kg, 0.004 7 mg/kg, 0.000 48 mg/kg and 0.004 7 mg/kg, respectively (Table 10). Table 11 summarizes the concentration of heavy metals in the analyzed Spirulina against international standards for levels of heavy metals in food.

Table 10: Heavy metals concentration in the locally available Spirulina products

Metals	Mean (SD)/mg/kg
Mercury	0.000 036 (0.000 008)
Lead	0.004 7 (0.01)
Cadmium	0.000 48 (0.0025)
Arsenic	0.004 6 (0.002)

Table 11: Concentration of heavy metals in the analyzed Spirulina against international standards for levels of heavy metals in food

Heavy metals	mg/kg			
	Cadmium	**Lead**	**Mercury**	**Arsenic**
Analyzed Spirulina	0.000 48	0.004 7	0.000 036	0.004 6
Codex	0.003-0.2	0.1-1	0.001-0.5	0.001-0.1
Australia New Zealand Food	0.005-0.1	0.1-0.5	0.5-1	1-2
European Communities Commission	1-3	1-3	0.1	0.1-0.3

Food Standards Australia New Zealand, 2015; CODEX Alimentarius, 2015; European Communities Commission, 2006

(ii) Serum toxicity markers

After the mice had been fed the experimental diets, a blood sample was taken and analyzed for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Cystatin C and Troponin I concentrations to evaluate the toxic effects induced by the diets on the internal body organs of the mice.

Serum AST concentration for control, test and standard group mice was 36 $\pm$ 0.70 U/L, 36 $\pm$ 0.11 U/L and 36.5 $\pm$ 0.14 U/L, respectively. Serum ALT concentration for control, test and standard group mice was 12 $\pm$ 0.12 U/L, 12.1 $\pm$ 0.70 U/L and 12 $\pm$ 0.42 U/L, respectively. Serum Cystatin C concentration for control test and standard group mice was 0.61 $\pm$ 0.28 mg/L, 0.65 $\pm$ 0.21 mg/L and 0.61 $\pm$ 0.11 mg/L, respectively. Serum Troponin I concentration for control, test and standard group mice was 0.004 $\pm$ 0.0028 ng/mL, 0.004 $\pm$ 0.004 ng/mL and 0.005 $\pm$ 0.004 ng/mL, respectively. Table 12 summarizes the measured concentration of serum toxicity markers in mice against the commonly defined normal toxicity markers ranges.

Statistical analysis showed that the serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Cystatin C and Troponin I concentrations was not statistically different between groups ($p > 0.05$). Figure 4 presents the serum concentration of toxicity markers measured from the mice consumed control, test and standard diets.

Table 12: Concentration of serum toxicity markers in mice against normal range values

Toxicity markers	Mean (SD)			
	Control	Test	Standard	[a] Normal range
AST (U/L)	36 (0.70)	36 (0.11)	36.5 (0.14)	35-140
ALT (U/L)	12 (0.12)	12.1(0.70)	12 (0.42)	10-35
Cystatin C (mg/L)	0.61 (0.28)	0.65 (0.21)	0.61 (0.21)	0.6-1
Troponin I (ng/mL)	0.004 (0.0028)	0.004 (0.000)	0.005 (0.004)	0-0.03

[a] Legacy laboratory services, 2020

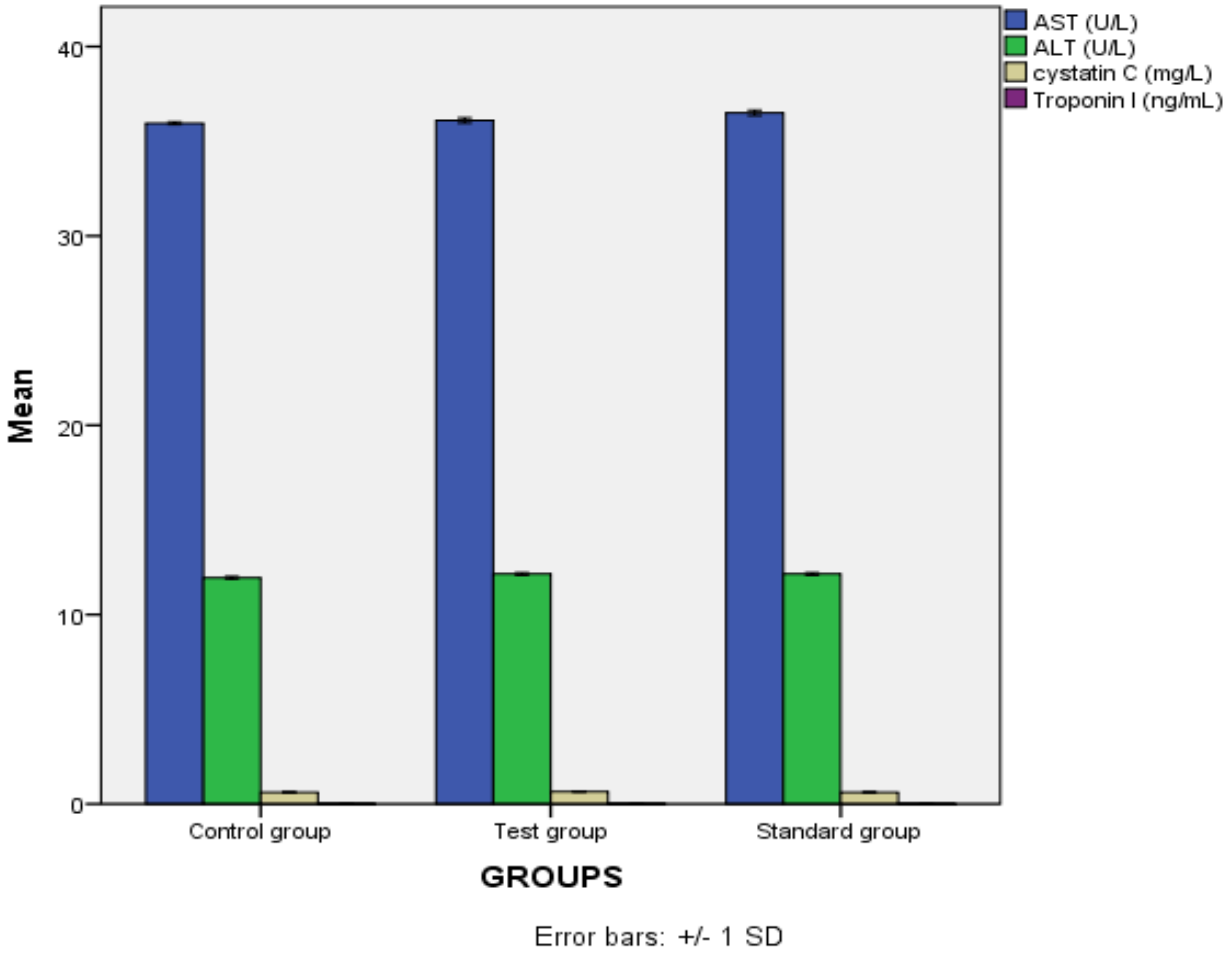

Figure 4: The measured serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Cystatin C and Troponin I concentrations were not statistically different between groups ($p > 0.05$). The data indicated that none of the diets had a unique effect on the internal organs of the mice

4.2 Discussion

4.2.1 Nutrient content of Spirulina

The World Health Organization's regional report showed that malnutrition remains the main cause of disease and death in Africa. Deficiencies such as protein, iron, zinc and vitamin A are the most prevalent ones in the region (WHO, 2017). Undernutrition occurs due to the lack of nutrients either as a result of poor diet or poor nutrient absorption from the diet (Akombi *et al.*, 2017).

Findings from the nutrient content analysis showed that a 100 g of locally found Spirulina contains a substantial amount of minerals, vitamins and protein in a concentration that is enough to meet the recommended daily allowance (RDA) for almost all age groups of individuals. Further, the concentration of nutrients happened to be higher in Spirulina than other foodstuffs.

The analyzed Spirulina contains 70 g/100 g protein which is higher than skimmed powdered milk (37 g/100 g), Whole soybean flour (36 g/100 g), Peanuts (26 g/100 g), chicken (24 g/100 g), fish (20 g/100 g), cow milk (3.3 g/100 g), goat milk (2.9 g/100 g), beef (18.5 g/100 g), goat meat (13.4 g/100 g), liver (19.9 g/100 g), eggs (12.1 g/100 g), termites (28.8 g/100 g) maize (5.9 g/100 g), wheat (10.5 g/100 g), beans (9 g/100 g) and spinach (3.3 g/100 g) (Dixit, 2018; Neumann *et al.*, 2002; Sanchez *et al.*, 2003).

The protein content in the analyzed Spirulina is enough to meet 100% of recommended daily allowance (RDA) for children under-five years, adults and pregnant and lactating women. As Protein-energy malnutrition (PEM) is a major public health concern in developing countries (WHO, 2018), considering Spirulina as a protein source of diet would help to eradicate the problem.

Results from this study showed that the analyzed Spirulina contain 82 mg/100 g iron content which is higher than the iron content in cow milk (0.04 mg/100 g), goat milk (0.04 mg/100 g), beef (3.2 mg/100 g), chicken (1.3 mg/100 g), goat meat (3.7 mg/100 g), fish (8.4 mg/100 g), offal (2.1 mg/100 g), liver (6.5 mg/100 g), eggs (1.54 mg/100 g), termites (2.5 mg/100 g), maize (2.9 mg/100 g), wheat (0.8 mg/100 g), beans (2 mg/100 g) and spinach (1.7 mg/100 g) (Neumann *et al.*, 2002).

The content of iron in the analyzed Spirulina is sufficient to meet 100% of recommended daily allowance (RDA) for children under five years, adults and pregnant and lactating women. Iron is a major component of numerous enzymes and protein that are required for normal biological functions of the body like DNA synthesis, energy production and oxygen transport; iron is required as a prosthetic group for myoglobin, hemoglobin, cytochromes and peroxidases biological activities (Abbaspour *et al.*, 2014). Inadequate dietary intake following depletion of the body's stores cause deficiency of iron; depletion of stored body iron causes impairment of red blood cell formation and hemoglobin synthesis, this, in turn, causes a health condition called anemia which is decreased oxygen-carrying capacity of the blood (Gebreweld & Tsegaye, 2018).

The content of zinc (85.4 mg/100 g) in the analyzed Spirulina is higher than the zinc contents in cow milk (0.31 mg/100 g), goat milk (0.22 mg/100 g), beef (6 mg/100 g), chicken (1.8 mg/100 g), fish (0.6 mg/100 g), eggs (1 mg/100 g), maize (0.33 mg/100 g), beans (0.3 mg/100 g) and spinach (0.7 mg/100 g) (Neumann *et al.*, 2002). The content of zinc in the analyzed Spirulina is sufficient to meet the recommended daily allowance (RDA) for children under five years, adults and pregnant and lactating women. Zinc is an essential micro-mineral required for various body functions such as structural, regulatory and catalytic functions (Deshpande *et al.*, 2013).

Calcium has important functions in the body, it is a major constituent of teeth and bones and also it has an essential role in the cell signaling pathways as a secondary messenger; when dietary calcium is inadequate, parathyroid hormone (PTH) and vitamin D controls the circulating calcium concentrations (Zhu & Prince, 2012). The analyzed Spirulina contain 1302 mg/100 g calcium which is higher than the calcium contents in cow milk (76 mg/100 g), goat milk (90 mg/100 g), beef (7 mg/100 g), chicken (13 mg/100 g), goat meat (17 mg/100 g), fish (37 mg/100 g), eggs (50 mg/100 g), maize (47 mg/100 g), beans (35 mg/100 g) and spinach (122 mg/100 g) (Neumann *et al.*, 2002). A daily intake of 1000-1300 mg calcium is required to meet the recommended dietary allowance (RDA) of adults, thus, 100 g of analyzed Spirulina is enough to meet the nutrition requirement.

The vitamin B_{12} content (3.99 μg/100 g) in the analyzed Spirulina is higher than vitamin B_{12} content in cow milk (0.29 μg/100 g), goat milk (0.05 μg/100 g), beef (2.4 μg/100 g), chicken

(0.23 μg/100 g), goat (1.2 μg/100 g), fish (0.6 μg/100 g) and eggs (1 μg/100 g) (Neumann *et al.*, 2002). The recommended dietary allowance (RDA) of vitamin B_{12} is between 0.5 and 2.8 μg/day, thus, a 100 g analyzed Spirulina is sufficient to meet the RDA for children under-five years, adults and pregnant and lactating women.

As it plays a vital role in the synthesis of fatty acids and energy productions, the metabolism of every cell in the body is dependent on vitamin B_{12}. Every minute the body produces millions of red blood cells. These cells cannot multiply properly without vitamin B_{12}. If the blood level of vitamin B_{12} is too low, the production of red blood cells reduces and anemia occurs (Stabler, 2013). Further, vitamin B_{12} enables the release of energy by helping the body absorb folic acid (Reynolds, 2006). Folic acid is also important for the production of red blood cells; red blood cells carry oxygen through the body; without enough folate, the person can develop a type of anemia called folate deficiency anemia, thus, similar to vitamin B_{12}, folate is important for the synthesis and repair of DNA and other genetic material (Haidar, 2010).

Comparing with previously published nutrient contents, Spirulina analyzed in this study contain the highest iron, calcium and protein concentrations far more than 21.98 mg/100 g iron, 0.28 mg/100 g calcium and 61.81 g/100 g protein reported by Ngo-Matipe *et al.* (2014) from Cameroon. Moreira *et al.* (2013) reported lower calcium (1.51 mg/100 g), phosphorous (0.8 mg/100 g) and zinc (3.54 mg/100 g) but higher iron (95.6 mg/100 g) concentrations from Spirulina samples obtained from Brazil than the contents found in this current study. Philippe *et al.* (2018) reported lower calcium (1002.06-1159.83 mg/100 g), phosphorous (233.38-392.51 mg/100 g), iron (23.38-25.13 mg/100 g), zinc (2.1-3.25 mg/100 g) and vitamin B_9 (17.25 μg/100 g) concentrations and higher vitamin B_{12} (10 μg/100 g) concentration from Spirulina biomasses obtained from different production ponds from Côte d'Ivoire. Seghiri *et al.* (2019) reported higher calcium (6000 mg/100 g) and protein (76.65 g/100 g) concentrations and almost similar iron (80.66 mg/100 g) content yet lower zinc (5 mg/100 g) content from Morocco than the values found in this current study. The calcium (70 mg/10 g), phosphorous (60 mg/10 g), vitamin B_9 (1 μg/10 g) and zinc (0.3mg/10 g) concentrations reported by Jung *et al.* (2019) was lower than the concentrations found in this study, however, this same study reported similar protein (55-70%) and higher vitamin B_{12} (20 μg/10 g) and iron (15 mg/10 g) concentrations.

The vitamin B_9 (50-300 μg/100 g) and vitamin B_{12} (100-300 μg/100 g) concentrations reported by Mohan *et al.* (2014) were higher than the concentrations found in this current study. Tang

and Suter (2011) reported higher concentrations of phosphorous (961 mg/100 g), iron (87.4 mg/100 g) and vitamin B_{12} (162 μg/100 g) but lower protein (63 g/100 g), calcium (468 mg/100 g) and zinc (1.54 mg/100 g) concentrations than the values found in this current study. Yin *et al.* (2017) reported higher calcium (1500 mg/100 g), iron (180 mg/100 g) and vitamin B_{12} (200 μg/100 g) concentrations but lower protein (14.6 g/100 g) content from Kenya than what is measured in this current study.

Ardiet and Weid (2005) reported lower (50 μg/100 g) vitamin B_9 and higher (150 μg/100 g) vitamin B_{12} concentrations than the values measured in the current study. Kumudha and Sarada (2015) reported higher vitamin B_{12} (220, 213 and 190 μg/100 g) concentrations from Spirulina extracted by using three different extraction methods in India than the vitamin B_{12} concentration analyzed in this current study. Sharoba (2014) reported higher vitamin B_9 (9920 μg/100 g) and vitamin B_{12} (175 μg/100 g) concentrations than the concentrations measured in this current study. Edelmann *et al.* (2019) reported higher B_9 (250-470 μg/100 g) and vitamin B_{12} (60-240 μg/100 g) concentrations from different commercial Spirulina biomasses from Asia than the values found in this current study.

Unal and Tokusoglu (2003) reported lower protein (61.3, 63.2, 64.4 g/100 g) and zinc (2.45, 2.57, 3.01 mg/100 g) concentrations and higher iron (90.1, 92.4, 103.6 mg/100 g) and phosphorous (703, 704, 802.7 mg/100 g) concentrations from the three different strains of Spirulina than the concentrations measured in this current study. Albert *et al.* (2012) reported higher iron (788.88, 683.33, 1666.6 mg/100 g) and zinc (298, 242, 216 mg/100 g) and lower calcium (3.1, 3.2, 3.15 mg/100 g) and phosphorous (375.5, 353, 20 mg/100 g) concentrations from Spirulina grown in three different production sites in Chad than concentrations found in this current study. Liestianty *et al.* (2019) reported lower protein (64.24 g/100 g) and higher calcium (1500 mg/100 g), phosphorous (1000 mg/100 g), iron (170 mg/100 g) and vitamin B_{12} (360 μg/100 g) concentrations from Indonesia than the concentrations analyzed in this current study.

Due to its higher production capacity, Spirulina is considered a good source of nutritional phenolic and flavonoid compounds than conventional plant-derived sources (Seghiri *et al.*, 2019). The measured total phenolic and total flavonoid contents in this current study were higher than 0.287 and 0.166 mg QE/g reported by Seghiri *et al.* (2019). Mane *et al.* (2019) reported higher phenolic (27.00 and 8.40 mg GAE/g) from aqueous and ethyl acetate extracts, respectively and also higher flavonoid contents (63.47 and 51.9221 mg QE/g) from chloroform

and methanol extracts, respectively. The total phenolic content reported from different strains of Spirulina (19.61, 39.33, 45.22, 48.93, 67.52 mg GAE/g) was higher than the total phenolic content analyzed in this current study (Aouir *et al.*, 2017).

The above discussion clearly showed that there is variation in the reported nutrient content of Spirulina from different studies conducted in a different parts of the world. The impact of environmental and growing conditions on the concentration of nutrients in Spirulina has been reported (Braga *et al.*, 2018; Fadel & Kamil, 2012; Fatemeh & Mohsen, 2016; Gatamaneni & Lefsrud, 2018; Sujatha & Nagarajan, 2013; Toyub *et al.*, 2011). Therefore, the exhibited nutrient content variation between the locally found Spirulina and other literature values could be due to variations in the geographical location where the Spirulina samples are taken from, growing conditions used to cultivate the Spirulina and also methods of extraction used to prepare Spirulina samples before nutrient content analysis.

4.2.2 Nutrient bioavailability of Spirulina

The fraction of an ingested nutrient that can be used by the body is obviously of major importance; composition analysis can give the information of the total amount of a nutrient in the diet but not the availability of the nutrient for absorption in the gut; factors such as processing conditions, the release of nutrient from the food matrix, possible interactions with other food components, the presence of anti-nutritional factors, the formation of stable compounds that are slowly metabolized, digestive enzymes in the intestine, adherence and uptake of nutrients by the intestine and transfer across the gut wall influence the availability of the nutrient for absorption (Jafari & Mcclements, 2017; Nguyen *et al.*, 2012).

Phytate, a natural product found in human diets has been described as an anti-nutrient because it exerts an inhibitory effect on the absorption of minerals during gastrointestinal passage by forming insoluble and indigestible complexes (Costa-Bauza *et al.*, 2012; Ma *et al.*, 2005). The concentration of phytate and minerals in the diets as well as the ratio of phytate/minerals determines the inhibitory effect of phytate on the bioavailability of minerals (Al Hasan *et al.*, 2016). In this study, very little (1.86 mg/100 g) concentration of phytate was measure as compared to rice (55 mg/100 g), rice boiling (35 mg/100 g), wheat flour (420 mg/100 g), wheat bread, white, baked (20 mg/100 g), whole wheat bread, baked (176 mg/100 g), corn flour (310 mg/100 g), baked corn-bread, unleavened (18 mg/100 g), millet (522 mg/100 g) and sorghum (427 mg/100 g) (Ma *et al.*, 2005). Besides, the phytate/iron (0.0019), phytate/zinc (0.0021) and

phytate/calcium (0.000086) molar ratios from Spirulina were below the critical levels at which the absorption of the mineral could be compromised. Moreover, the ratio of mineral/mineral may affect the absorption of minerals from the diet (Nguyen *et al.*, 2012). The computed iron/zinc, zinc/iron and calcium/ phosphorous molar ratios from Spirulina were 1.14, 0.88 and 1.6, respectively. The values were below the critical levels at which the absorption of minerals could be compromised. Therefore, the chemical interaction between phytate and essential minerals as well as between minerals does not have an inhibitory effect on the absorption of minerals from Spirulina.

Oxalate is also an anti-nutritional factor present in the diet that hinders nutrient absorption (Kasimala *et al.*, 2018). Oxalate binds to essential minerals in the digestive tract and forms insoluble salts, which make minerals unavailable for absorption; besides, if the insoluble salts created in the digestive system are not excreted in the urine, they will be accumulated in the kidney and cause kidney stone (Nile & Park, 2014), thus, the adverse effect of oxalate is not only hindering nutrient absorption but it also has negative health affect. The inhibitory effect of oxalate on the absorption of minerals from Spirulina is none because Spirulina is free from oxalate (Gutiérrez-Salmeán *et al.*, 2015).

Having high tannin concentration makes the food be of low nutritional value because of tannin's inhibitory effect on nutrient absorption (Kim & Miller, 2005). Similar to other anti-nutritional factors, tannin inhibits nutrients absorption through insoluble tannin nutrient complex formation (Delimont *et al.*, 2017). However, due to the undetectable level of tannin in Spirulina (Mane *et al.*, 2019; Seghiri *et al.*, 2019), its inhibitory effect on the absorption of nutrients from Spirulina is minimal.

The rate and extent to which nutrients are absorbed and become available at the site of action is bioavailability as defined by the United States Food and Drug Administration (FDA) (Shi & Le Maguer, 2000). Bioavailability is a combination of bioactivity and bio-accessibility, where bio-accessibility refers to the release of nutrients from the food matrix, transformations during digestion and transport across the digestive epithelium, whereas bioactivity includes uptake into tissues, metabolism and physiological effects (Holst & Williamson, 2008). Due to practical and ethical difficulties in terms of measuring bioactivity, the fraction of a given nutrient that reaches the systemic circulation and available for absorption is considered bio-accessible, but not necessarily bioactive (Holst & Williamson, 2008).

The microstructure of processed food or the matrix of natural food can favor or hinder the final uptake of nutrients in the gut (Aguilera, 2018). Natural foods are organized hierarchically from molecules into assemblies, organelles, cells and tissues; the spatial arrangement of elements in food and their interactions at a lower level (below 100 μm) is defined as food microstructure (Aguilera, 2018). The typical food microstructure includes starch granules, cell walls, proteins, fat crystals, water and oil droplets and gas bubbles (Parada & Aguilera, 2007). Nutrients are contained in a larger continuous medium that may be natural or a microstructure which is produced by processing and their interaction with the components and the structure of the medium at different length scales is the major concept of food matrix (Parada & Aguilera, 2007). The arrangement of nutrient could be either as individual molecules bound to plant organelles or as a macromolecular matrix of swollen starch granules and protein; nutrients need to be released from the natural cellular compartments or assemblies produced during processing to be available for absorption by the gut; thus, the release of intracellular nutrients is influenced mainly by the digestibility of the microstructural elements in food such as cell wall (Parada & Aguilera, 2007).

The cell wall of Spirulina is made from four layers measuring 10 to 15 nm thickness (Eykelenburg, 1977). Being composed of soft mucopolysaccharides (long linear polysaccharides consisting of repeating disaccharide units) and lack of cellulose in the Spirulina cell wall makes it easily digested and assimilated (Sharoba, 2014). Cellulose is a tough, fibrous and water-insoluble polysaccharide that plays an integral role in keeping the structure of plant cell walls stable; cellulose chains are arranged in microfibrils or bundles of polysaccharides that are arranged in fibrils (bundles of microfibrils), which in turn make up the plant cell wall (Zeng *et al.*, 2017).

The bioavailability of nutrients can be determined by methods that involve a human or animal (*in vivo*) or simulated experiments performed in a laboratory (*in vitro*). Since *in vivo* methods provide direct data of bioavailability they have been used for a great variety of nutrients; usually, a response is measured after consumption of a pure nutrient (natural or synthetic) by living beings, either humans (most common) or animals and compared to an equivalent nutrient dose found in a food source (Yeum & Russell, 2002). Due to ethical restrictions and abiding by severe protocols when humans and/or animals are used in biological research are limiting factors to conduct *in vivo* studies (Hof *et al.*, 2000), *in vitro* methods such as polarized human

colon carcinoma cells line Caco-2 cells are being extensively used at present (Verwei *et al.*, 2003).

In this study, *in vivo* method which involved experimental mice was used to evaluate the nutrient bioavailability of experimental diets. Since the control diet had significantly lower nutrient content, control group mice had lower nutrient intake. On the other hand, the test and standard diets had equivalent nutrient content and therefore, the intakes of nutrients between these two groups were similar.

In the nutrient bioavailability experiment, the concentration of nutrient in the feces reflects the fraction of ingested nutrient which is not absorbed (Safwat *et al.*, 2015). The results showed that fecal nutrient content was statistically different between groups. However, the percentage of nutrient excreted from control diet was higher than that of test and standard diets and the percentage of nutrients excreted from test and standard diets were similar (but not statistically) except for vitamin A. The higher the nutrient excreted, the lower nutrient absorption.

The gastrointestinal tract (GIT) is a series of organs joined from the mouth to the anus; the organs that make up the GI tract are the mouth, esophagus, stomach, small intestine, large intestine and anus. The liver, pancreas and gallbladder are also the solid organs of the digestive system (Gropper *et al.*, 2009). The small intestine absorbs most of the nutrients in the food and the circulatory system passes the absorbed nutrients onto the liver, which stores, processes and delivers the absorbed nutrients to the rest of the body when needed (Gropper *et al.*, 2009). Thus, nutrients that are reached to the liver tissue considered bioavailable in this context.

The findings showed that liver tissue nutrient content from control group mice was statistically lower than that of test and standard group mice, this indicated that the control diet had lower nutrient bioavailability than the other two experimental diets. On the other hand, the bioavailability of calcium, phosphorous, zinc and vitamin B_9 from the test diet was higher than both control and standard diets. Also, the bioavailability of iron and vitamin B_{12} from the test diet was higher than the control diet but similar to the standard diet. However, the bioavailability of vitamin A from the test diet was lower than the standard diet. This indicated that the ability of calcium, phosphorous, zinc and vitamin B_9 from the test diet to reach the target tissue was better than both control and standard diets.

The findings from this current study were in agreement with several other studies on the topic. For instance, after assessing the effect of Spirulina, calcium carbonate and high calcium milk supplementations on the serum calcium and bone mass density of rats, Ekantari *et al.* (2016) reported that the calcium of Spirulina has a higher bioavailability than those of calcium carbonate and high calcium milk. Another study that compared the bioavailability of vitamin B_{12} from supplementation of Spirulina and pure cyanocobalamin in vitamin B_{12} deficient rats revealed that the vitamin B_{12} from Spirulina was absorbable, however, the level of absorption from Spirulina was lower than that of cyanocobalamin (Berg *et al.*, 1991). Also, Madhubalaji *et al.* (2019) reported that supplementation of Spirulina in the diet of vitamin B_{12} deficient rats leads to the normalization of vitamin B_{12} deficiency-induced circulatory and functional biomarkers, indicating the vitamin B_{12} from Spirulina was absorbable and able to improve the systemic vitamin B_{12} status. Similarly, the study administered a daily supplementation of 10 g Spirulina along with local diet to the intervention group children and only local diet to the control group children for 30 days reported that the weight-for-age Z scores and weight-for-height Z scores increased quickly and significantly in the intervention group, the results were indicated that supplementation of Spirulina improved the nutritional status of children through improving systemic nutrient level (Matondo *et al.*, 2016). Also, a cohort study administered 1500 mg/d Spirulina supplementation to the intervention group of pregnant women and iron and folic acid supplementation to the control group of pregnant women reported that the hemoglobin level of intervention group pregnant women was significantly higher than that of iron and folic acid group. Thus, the study concluded that Spirulina is a good option to control iron deficiency anemia and maternal mortality (Niang *et al.*, 2017). Furthermore, studies conducted to assess the impact of Spirulina supplementation on the nutritional status and health conditions of human and animal subjects proved that Spirulina contains absorbable nutrients that can improve the nutritional status and health conditions of consumers (Dixit, 2018; Marcel *et al.*, 2011; Ngo-Matipe *et al.*, 2014; Simpore *et al.*, 2007; Sop *et al.*, 2015; Yu *et al.*, 2018).

As previously published studies reported the higher bioavailability of Spirulina nutrients, the findings from this study also revealed that Spirulina nutrients are comparably bioavailable with the conventional nutritional supplements.

4.2.3 Safety of Spirulina

Diet, as well as nutraceutical products, exert a direct impact on health and incidence of many chronic diseases (Elliott & Ong, 2002). Toxic concentrations of certain food compounds can

adversely affect human health, while other bioactive food compounds can modulate the development of chronic illnesses, like chronic liver diseases (Chalabi *et al.*, 2008). Heavy metals have been shown to cause acute as well as chronic poisoning in humans and other experimental animals in higher dose consumptions (Engwa *et al.*, 2016). Metallic toxic substances are intrinsic components of the environment and they are encountered in numerous occupational and environmental circumstances and the impact of these toxic agents on human health is a public health concern due to the ubiquity of exposure (Jaishankar *et al.*, 2014). With the increasing applications of different variety of metals in industries such as pharmaceuticals, paper and pulp preservatives, agriculture industry and chlorine and caustic soda production industry, public health problem from toxic metal pollution of the environment is arising (Jaishankar *et al.*, 2014). The chemical reactivity of the heavy metal ions with cellular structural proteins, enzymes and membrane system determines their toxicity; organs that accumulate the highest concentrations of the metal *in vivo* such as the brain, liver, kidney and lung are target organs of specific metal toxicities and the toxicity of metals often depends on the route of exposure and the chemical compound of the metal i.e. its valiancy state, volatility, lipid solubility and so on (Jaishankar *et al.*, 2014).

Ingestion of large amounts of soluble inorganic arsenic can have immediate toxic effects and leads to gastrointestinal symptoms such as severe vomiting, disturbances of the blood and circulation, damage to the nervous system and eventually death; if it is not death, a large dose of arsenic ingestion may reduce blood cell production, break up red blood cells in the circulation, enlarge the liver, color the skin, produce tingling and loss of sensation in the limbs and cause brain damage (Engwa *et al.*, 2016). Dietary exposure to cadmium leads to kidney, liver, lung and bone damage (Järup, 2003). Mercury is considered the most toxic metal in the environment and dietary ingestion of mercury is the main source of exposure (Jaishankar *et al.*, 2014). Symptoms such as tremors, emotional lability, insomnia, memory loss, neuromuscular changes and headaches as well effects on the kidney and thyroid are the reported symptoms from ingestion of elementary mercury (Järup, 2003).

There are international standards for levels of heavy metals in foods including the Codex General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995), the US Food and Drug Administration (FDA), Australia New Zealand Food standard code and European Communities Commission (EC). However, they only include a few types of food

items and don't have a standard level for microalgae products. Also, there is limited information on national standards for levels of heavy metals in food.

The maximum cadmium level in the food according to CODEX is between 0.003 mg/kg for natural mineral water and 0.2 mg/kg for leafy vegetables. The maximum level for Lead is between 0.1 for fruits and 1 for canned fruit products. The maximum level for mercury is between 0.001 mg/kg for natural mineral water and 0.1 mg/kg for food-grade salt and 0.5 mg/kg for fish. The maximum level for arsenic is 0.001 mg/kg for natural mineral water and 0.1 mg/kg for edible fat and oil (CODEX Alimentarius, 2015).

According to Australia New Zealand Food standard code, the maximum level of arsenic is between 1 mg/kg for seaweeds and 2 for fish. The maximum level of cadmium is between 0.05 mg/kg for meat and 0.1 for leafy vegetables. The maximum level of lead is between 0.1 mg/kg for vegetables and 0.5 mg/kg for fish and fruits. The maximum level of mercury is between 0.5 mg/kg and 1 mg/kg for fish and fish products (Food Standards Australia New Zealand [FSANZ], 2015).

According to the European Communities Commission (EC), the maximum level of lead is between 0.01 and 0.5 mg/kg for different food items and 1-3 mg/kg for food supplements. The maximum level of cadmium level is between 0.05 to 1 mg/kg for various food items and 1-3 mg/kg for food supplements. the maximum level of mercury is between 0.5 to 1 mg/kg for different food items and 0.1 mg/kg for food supplements. The maximum level of arsenic is between 0.1 and 0.3 mg/kg for different food items (European Communities Commission [EC], 2006).

Comparing with the international standards for levels of heavy metals in food, the concentration of mercury (0.000036 mg/kg), lead (0.0047 mg/kg), cadmium (0.00048 mg/kg) and arsenic (0.0047 mg/kg) measure in locally available Spirulina was very little. Thus, the findings from this study revealed that heavy metal induced contamination is not a concern from Spirulina consumption. Besides, Al-Homaidan (2006) reported little concentration of mercury (0.008 mg/kg), lead (0.109 mg/kg), cadmium (0.031 mg/kg) and arsenic (0.002 mg/kg) from Saudi Arabian Spirulina. Moreover, small concentration of arsenic (0.006-0.578 mg/kg), lead (0.1-1.206 mg/kg), cadmium (0.003-0.069 mg/kg) and mercury (0.002-0.028 mg/kg) was reported from commercial Spirulina products (Al-Dhabi, 2013).

Biomarkers have been used in the prediction of organ dysfunction (Ramaiah, 2007). A biomarker can be any hormone, enzyme, antibody or other substances detected in urine, blood,or other body fluids or tissues which may serve as a sign of a disease or other abnormality, therefore, increased concentration of serum biomarkers indicates the occurrence of disease and abnormalities (Ramaiah, 2007). Among several biomarkers, troponin I is the biomarker of choice for the detection of cardiac injury due to its sensitivity (Babuin & Jaffe, 2005). Troponin or troponin complex is a complex of three regulatory proteins (troponin I, T and C) that are integral to muscle contraction in skeletal muscle and cardiac muscle; circulating cardiac troponin concentrations have become an increasingly important biomarker for the detection of myocardial injury in many cardiovascular diseases, thus, elevated troponin levels are highly specific for cardiac injury (Babuin & Jaffe, 2005).

Aminotransferases are a group of enzymes that participate in the process of gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively; Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are excellent and commonly used biomarkers of hepatocellular injury (Goorden *et al.*, 2013). Aspartate aminotransferase (AST) present in cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes and red cells, thus, it is less sensitive and specific to evaluate liver function; however, alanine aminotransferase (ALT), a cytosolic enzyme is found in its highest concentrations in the liver and is more specific to the liver (Pratt & Kaplan, 2000).

Cystatin C (cysC) is a low molecular mass protein produced by most nucleated cells; this protein is freely filtered at the glomerulus and practically completely reabsorbed by tubular cells; the production rate of cystatin C is remarkably constant and therefore, its plasma concentration has been proposed as a suitable marker of glomerular filtration rate (Filler *et al.*, 2005). Serum cystatin C (CysC) has been used as a more sensitive and more accurate marker of enzymatic measurement for renal dysfunction (Christensson *et al.*, 2003; Leelahavanichkul *et al.*, 2014).

There is a lack of standardized values among laboratories to determine the elevated serum toxicity marker levels (Galteau *et al.*, 2001), despite that there are commonly defined normal ranges used by different laboratories. For instance, the normal ranges published by the Legacy laboratory (2020) is 35-140 U/L for aspartate aminotransferase, 10-35 U/L for alanine aminotransferase, 0.6-1 mg/L for cystatin C and 0-0.3 ng/mL for troponin I. the serum

concertation of biomarkers measured in this current study agreed with the normal range defined by Legacy Laboratory. Besides, the alanine aminotransferase concentration found in this study was in agreement with the previously reported 16.05 and 16.7 U/L concentrations from the low and high dose of Spirulina consumption in rats (Sixabela *et al.*, 2011). Moreover, Naidu *et al.* (1999) evaluated the toxic effect of phycocyanin, a natural colorant from Spirulina consumption in rats and reported no induce symptoms of toxicity nor mortality in rats. Besides, in the United States, the FDA (Food and Drug Administration) classified Spirulina as GRAS (generally recognized as safe). Moreover, according to the classification of the Center for Food Safety and Applied Nutrition (CFSAN), Spirulina biomass is classified as 'other dietary supplement without toxicological effects'. In addition to the United States FDA, Health Canada, Food Standards Australia New Zealand (FSANZ) and Brazilian Health Regulatory Agency have granted permission for using Spirulina products as a novel food ingredient (García *et al.*, 2017; Navacchi *et al.*, 2012).

In general, the concentration of heavy metals in the analyzed Spirulina, as well as the concentration of serum toxicity markers measured from the mice, consumed Spirulina revealed the locally found Spirulina has no toxic effect in mice. However, the safety of Spirulina which is collected directly from Lake Victoria needs to be evaluated since the lake might be contaminated with different heavy metals and microcystins. A recent study reported the presence of microcystin and eutrophication challenges in Lake Victoria (Otoigo *et al.*, 2020). The World Health Organization (WHO) provisional microcystins level in the drinking water is 1 µg/L. However, the microcystins level of 3.44 µg/L was reported from the drinking water supplied from Lake Victoria (Otoigo *et al.*, 2020). Further, WHO provisional microcystins in recreational water are 20 µg/L, however, beaches of Lake Victoria had microcystins levels ranging between 10.16 and 94.34 µg/L (Otoigo *et al.*, 2020). Besides, contaminations of heavy metals such as lead, arsenic and cadmium was reported from Lake Victoria (Machiwa, 2004; Makundi, 2001).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The nutrition profile of locally available Spirulina products suggests superiority against commonly utilized food commodities. In this study, the concentrations of various nutrients in locally found Spirulina are enough to meet the recommended daily allowance (RDA) for almost all age groups of individuals, confirming its importance in human nutrition. Currently, the literature suggests that Spirulina is a generally safe food supplement with no significant side effects. Our study on mice also indicates that locally found Spirulina is toxicologically safe, suggesting its potential expanded application in human diets. In fact, human attitudes on foods are constantly changing globally. Given the nutrition information provided in this study, consumers in East Africa could benefit from Spirulina nutrition if the food industry sector in the region can invest in food product development and designs targeting palatable Spirulina levels in common diets and food forms. Of course, consumer studies to understand overall acceptance of such products will be required.

5.2 Recommendations

Product and consumer understanding on various food forms (e.g. biscuits, spaghetti, smoothies, etc.) developed with Spirulina is required. This will ensure access to adequate nutrition provided by Spirulina as a possible and socially relevant intervention for mitigating malnutrition in the region.

Evidence-based efficacy studies required to address possible future clinical applications in nutritionally vulnerable cohorts such as children.

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APPENDICES

Appendix 1: Evidence of ethical clearance for the study

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC00026 **23TH JANUARY 2020**

Study Title: Establishing scientific evidence on the potential of microalgae in improving nutrition in East Africa

Study Area: EAST AFRICA

PI Name: FevenTezera Damessa

Co-Investigator:

Institutions: **NM-AIST** School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 21ST JANUARY 2020

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute for Medical Research, and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Medical Research and Ministry of Health Community Development Gender Elderly and Children.

Duration of Study Renewal: Subject to Renewal within ONE YEAR

Span From: 22ND JANUARY 2021.

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Mr. Simon Njeya
Secretary
KNCHREC

Chairperson
KNCHREC

RESEARCH OUTPUTS

(i) Journal papers

Damessa, F. T., Chacha, M., Vianney, J. M., & Raymond, J. (2021). *In vivo* evaluation of *Spirulina platensis* for nutrient bioavailability in mice. *Applied Biological Research, 23*(1), 37-44. DOI: 10.5958/0974-4517.2021.00005.7

Damessa, F. T., Chacha, M., Vianney, J. M., & Raymond, J. (2020). Nutritional values of *Spirulina platensis* biomass cultivated in East Africa. *International Journal of Biosciences, 16*(6), 121-128. http://dx.doi.org/10.12692/ijb/16.6.121-128

Damessa, F. T., Chacha, M., Vianney, J. M., & Raymond, J. (2021). Measuring Serum Toxicity Markers to Evaluate the Safety of Commercially Available Spirulina Products in Mice. *Current Research in Nutrition and Food Science, 9*(1), 1-7. https://bit.ly/394Qo1f

(ii) Poster presentation