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Development of a natural product rich in bioavailable OMEGA-3 DHA and EPA using locally available ingredients in Tanzania

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DEVELOPMENT OF A NATURAL PRODUCT RICH IN BIOAVAILABLE OMEGA-3 DHA AND EPA USING LOCALLY AVAILABLE INGREDIENTS IN TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Poor mental health remains a serious public concern worldwide. The most vulnerable individuals are children and adolescents in developing countries. Nutritional deficiency of Omega-3 DHA and EPA has long been recognized as a major contributing factor for mental health illnesses. Provision of ready-to-use natural product rich in preformed Omega-3 DHA could address this problem. However, most commonly used products are expensive and contain less or no preformed Omega-3 DHA, making them less suitable for prevention of mental illnesses in resource-poor countries. The main objective of this study was to develop a natural product rich in preformed Omega-3 DHA and EPA from locally available ingredients. Linear programming (LP) was used to formulate a natural product rich in preformed Omega-3 DHA and other essential nutrients using locally available ingredients other than fish and dairy products. Laboratory analysis was then performed to validate the nutritional value of the LP-formulation using standard analytical methods. The relative difference between the LP tool calculated values and the laboratory-analysed values were calculated. Sensory testing was also done to evaluate consumer acceptance of the final product. Optimal formulation contained 220 mg of preformed Omega-3 DHA+EPA, enough to meet the RDI for children aged 2-10 years. The LP analysis further showed that the cost of present product is USD 0.15/100 g, which is 50% lower than that of Plumpy'nut. Laboratory analysis revealed similar results as that of LP at P=0.05. These findings indicate that ready-to-use natural food rich in preformed DHA and EPA can be developed from locally available ingredients.

Keywords: microalgae, mental health, preformed Omega-3 DHA, nutrition, nutrient-based intervention, linear programming.

DECLARATION

I, **Christina N. Charles** do hereby declare to the Senate of 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.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a dissertation entitled: **"**Development of a Natural Product Rich in Bioavailable Omega-3 DHA and EPA Using Locally Available Ingredients in Tanzania" in partial fulfillment of the requirements for the Degree of Masters of Life Science of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This work is dedicated to the Almighty God for His protection and guidance throughout my academic life. This work is also dedicated to my lovely husband Dr. Jofrey Raymond and our beautiful daughter Victoria for their unconditional love and care during my studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADHD	Attention Deficit Hyperactivity Disorder
ALA	Alpha Linolenic Acid
BBB	Blood Brain Barriers
DHA	Docosahexaenoic Acid
DIAAS	Digestible Indispensable Amino Acid Score
EPA	Eicosapentaenoic Acid
FAO	Food and Agriculture Organization of the United Nations
GAIN	Global Alliance for Improved Nutrition
GLM	Generalized Linear Model
LP	Linear Programming
NGOs	Non-Governmental Organizations
PDCAAS	Protein Digestibility Corrected Amino Acid Score
RDI	Recommended dietary intake
RUFs	Ready-to-Use Foods
SDGs	United Nations' Sustainable Development Goals
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
WFP	World Food Programme
WHO	World Health Organization of the United Nations
WTP	Willingness to Pay

CHAPTER ONE

1.1 Background of the problem

Mental health problems represent a significant public health concern worldwide (World Bank Group/ WHO, 2016). The problem accounts for 13% of the overall global burden of disease. It also accounts for more than one third of adult disabilities (Vigo *et al*., 2016). The most at risk individuals are young children, adolescents and women of the reproductive age. Briefly, mental illness is a diagnosable mental health disorder, which is characterized by alterations in thinking, mood, or behaviors associated with distress and or impaired functioning. The problem is more prevalent in developing countries, especially in households that are in resource-poor environments (World Bank Group/ WHO, 2016). In Tanzania for instance, the regional analysis has shown that the average dietary intake of DHA is 57.1 mg/day, which groups the country among countries with the lowest DHA dietary intake in the world (Forsyth *et al*., 2016). Nutritional deficiency has long been recognized as a major contributing factor of mental disorders in resource-poor countries (Korn, 2016). The most common nutritional deficiencies seen in patients with mental disorders are Omega-3 fatty acids, B vitamins, minerals and amino acids, all of which are precursors to neurotransmitters (Rao *et al*., 2008).

Since nutrition is clearly linked with mental health, the field of nutrition has recently received attention as a sustainable approach to both the prevention and management of mental health disorders in low and high income countries (Marx *et al*., 2017; Ramakrishnan *et al*., 2009). Convincing data suggest that nutrient-based supplements might provide many neurochemical modulatory activities that are beneficial in the prevention and management of mental health disorders (Forsyth *et al*., 2017). Because of that, there are a number of nutrient-based interventions that are currently being implemented especially, in developed countries (Panse & Phalke, 2016). Long-chain Omega-3 fatty acids supplementation, especially docosahexaenoic acid (DHA) is an example of the available nutrient-based interventions for prevention and or management of mental disorders (Stark *et al*., 2016). The assertion is based on the clinical trials that have proved the usefulness of omega-3 DHA in prevention and management of psychosis, bipolar depression, major depression and post-traumatic stress disorder (Sun *et al*., 2018; Thome Research Inc., 2009).

Omega-3 fatty acids, especially docosahexaenoic acid (DHA) have a wide range of scientifically established health benefits attributed to their consumption. Docosahexaenoic

acid is highly concentrated in the brain, and is absolutely essential for proper brain development and cognitive function of infants and young children (Markhus *et al.*, 2015). Docosahexaenoic acid is crucial at all stages of life but particularly in pre conception, pregnancy and childhood years. Brain development continues through childhood and early adolescence, with cerebral volume reaching 95% of its peak by six years of age and reaching its maximum between 10 and 15 years of age (Weiser *et al*., 2016). Studies have reported that poor DHA status may affect brain development as well as the cognitive abilities of children (Forsyth *et al*., 2016).

Functional foods that are rich in alpha-linolenic acid (ALA) are being promoted as the reliable alternative source of DHA. The assertion is based on a scientific fact that dietary ALA can be converted into DHA within the body (Dewick, 2009). Furthermore, the ingredients that are rich in ALA are readily available in the global market. Some of the well-studied ALA-rich ingredients available in the market include soybeans, chia seeds, flaxseed, walnut and canola (Lenihan-Geels *et al*., 2013). Because of the reported health benefits of omega-3 fatty acids, there are a number of omega-3 products on the global market that are being advertised as foods for brain development and cognitive function (Panse & Phalke, 2016). One should however, note that omega-3 DHA cannot be synthesized *de novo* in humans, and therefore, must be obtained directly from the diet or synthesized within the body from ALA (Sun *et al*., 2018). However, conversion of ALA into DHA is almost negligible. Therefore, consuming ingredients that are rich in omega-3 ALA may not confer any physiological benefit to the brain. Therefore, consuming ingredients that are rich in omega-3 ALA may not confer any physiological benefit to the brain.

Currently, fish oils represent the most prominent dietary sources of omega-3 DHA. However, according to Lane and colleagues, there are several limitations to relying fish oils as a source of supply of omega-3 DHA (Lane *et al*., 2014). Some of these limitations include the undesirable odors, flavors, and tastes of fish oils, which discourage consumers from consuming them in their pure forms, and neither is traditional supplementation much appreciated. Furthermore, some fish contain high levels of methyl-mercury, which creates a risk of mercury poisoning to consumers. Furthermore, some fish are likely to contain high levels of methyl-mercury, creating a risk of mercury poisoning. In addition, environmental contaminants such as dioxins and polychlorinated biphenyls have been found in fish oils, which may dissuade its use. In addition, fish oil has already reached maximum global

production and its stock is decreasing throughout the world, thus may not be a sustainable source of DHA, especially in resource-poor countries. This calls for development of products or formulations that are rich in preformed DHA and other health promoting compounds from other sources.

Consumption of products that are rich in preformed DHA and cofactors could ensure bioavailability of dietary omega-3 DHA in the body. Tanzania is among the global baskets of ingredients (e.g. microalgae) that are naturally packed with preformed omega-3 DHA and other health promoting compounds that are mostly absent in typical African diets for children and the general population (Rajauria *et al*., 2015). Unlike other countries, these natural ingredients are not yet fully utilized for human consumption in Tanzania, neither have the guidelines for omega-3 DHA intake been developed. This underscores the need for formulating a natural product from locally available ingredients that are rich in preformed omega-3 DHA and other essential nutrients and make them available for human consumption in the country.

So far, there is no convenient optimal formulation rich in preformed DHA and EPA for human consumption in Tanzania and East Africa at large despite the presence of microalgae, which is a cheap and sustainable natural source of omega-3 DHA and other health promoting compounds. Majority of omega-3 products that are found in the market are ALA, whose conversion in the body may not achieve the recommended daily intake of DHA. Since food companies know that only few consumers understand the difference between ALA and DHA omega-3, they sucker people into buying ALA products by advertising them as omega-3. Under this circumstance, the status of DHA among the consumers is likely to be very poor, especially among vulnerable individuals in developing countries (Forsyth *et al.*, 2016). Poor DHA status may affect brain development as well as the cognitive abilities of infants and young children. Thus, bioavailable omega-3 DHA should be included in the diets for infants and children to ensure optimal brain and cognitive development (Sun *et al*., 2018). Maintaining individual's mental health is important to improve personal life values, to reduce medical costs and other social expenses that are incurred in dealing with mental disorders, and to enhance national competitiveness (World Bank Group/ WHO, 2016).

Optimal use of locally available ingredients other than fish oil can ensure dietary adequacy of bioavailable omega-3 DHA and other health promoting compounds among the vulnerable individuals in developing countries like Tanzania. This can be achieved using linear

programming (LP) techniques. Linear programming is a mathematical tool which allows generation of optimal solutions that satisfy nutritional and other constraints at once (Dibari *et al.*, 2012). It has been used in optimizing the ratio of omega-3 and omega-6 fatty acids for health promoting diets (Cahyaningrum *et al.*, 2016). Linear programming can therefore, be used in formulation of ready to use natural products that are rich in preformed DHA and other health promoting compounds. The technique can also be used to inform regulatory authorities to design appropriate policies and dietary guidelines for omega-3 DHA that are currently missing in developing countries. The aim of this study was to formulate a ready to use natural product rich in preformed omega-3 DHA and other essential nutrients from locally available ingredients other than fish oil and dairy sources using linear programming. Optimized formulation can be used to prevent mental disorders associated with poor nutrition among individuals in Tanzania and other countries facing similar challenge.

1.2 Statement of the problem

The research problem being addressed in this study is the development of a convenient natural product rich in bioavailable omega-3 DHA and EPA fatty acids. So far, there is no convenient natural product, which is rich in bioavailable DHA in Tanzania and other similar places in sub-Saharan Africa. Majority of omega-3 products that are found in the market are ALA, whose conversion in the body may not achieve the recommended daily intake of DHA. Since only few consumers understand the difference between ALA and DHA omega 3, companies use this advantage to sell ALA products by advertising them as omega 3. Under this circumstance, the status of DHA among the consumers is likely to be very poor, especially among vulnerable individuals in developing countries (Forsyth *et al.*, 2016). Poor DHA status may affect brain development as well as the cognitive abilities of infants and young children. Thus, bioavailable omega-3 DHA should be included in the diets of infants and children to ensure optimal brain development and cognitive performamnce (Sun *et al*., 2018).

1.3 Rationale of the study

Omega-3 DHA is vital at all stages of life but, particularly, in pre-conception, pregnancy and childhood years. Low DHA content can lead to poorer brain development and cognitive performance of a growing child. Thus, it is vital that pregnant and lactating women as well as infants consume sufficient preformed DHA to support mental health. Maintaining

individual's mental health is important to improve personal life values, to reduce medical costs and other social expenses that are incurred in dealing with mental disorders, and to enhance national competitiveness (World Bank Group/ WHO, 2016).

1.4 Objectives

1.4.1 General objective

The main objective of this study was to develop a convenient natural product rich in preformed omega-3 DHA and EPA fatty acids, and cofactors for conversion of ALA into DHA.

1.4.2 Specific objectives

(i) To identify suitable local ingredients for developing omega-3 DHA natural product

(ii) To design optimal formulation for developing a natural product rich in preformed omega-3 DHA and its absorption cofactors

(iii) To develop natural product prototype rich in bioavailable omega-3 DHA and cofactors

(iv) To evaluate consumer acceptance and willingness to pay for the final product

1.5 Hypothesis

Local ingredients other than fish oil (if optimized) can ensure dietary adequacy of bioavailable omega-3 DHA and EPA among the vulnerable individuals in developing countries

1.6 Significance of the study

The natural product from this research study will improve mental health of consumers, especially children and enable them to realize their own potential, cope with the normal stresses of life, work productively and fruitfully, and make a significant contribution to their community at large. Similarly, this natural product will contribute significantly to the current global efforts of preventing maternal and child mental health illness. In a long-term plan, this product will be commercialized, which in turn will create direct employment and increase the value of indirect jobs in Tanzanian and East Africa in general.

1.7 Delineation of the study

This study was conducted to develop a natural product rich in preformed omega-3 DHA and other essential nutrients from locally available ingredients in Tanzania. Linear programming techniques was used to develop this natural product rich in preformed omega-3 DHA and other essential nutrients from identified local ingredients. Laboratory analysis and sensory evaluation were performed to validate nutritional and sensory qualities of the product. Findings from the present study show that acceptable and convenient ready-to-use natural product rich in preformed DHA and other essential nutrients can be developed from locally available ingredients in Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global state of mental health disorders

Poor mental health remains a public concern worldwide. The global burden of mental health problems is high, and is predicted to rise in the near future. The most at risk individuals are children, adolescents and women of reproductive age in developing countries. The 2016 World Bank Group and World Health Organization estimates (World Bank Group/ WHO, 2016) indicate that more than 10% of the world's population is affected by mental disorders and that 20% of children and adolescents suffer from some type of mental disorder. In fact, mental disorders account for 30% of non-fatal disease burden worldwide and 10% of overall disease burden, including death and disability. Low- and middle-income countries contribute about 80% of individuals who are likely to experience an incidence of a mental disorder in their lifetime. Generally, mental illness include depression, bipolar disorder, schizophrenia, eating disorders and anxiety disorders, attention deficit disorder, autism and addiction. But, the most common childhood mental disorders are anxiety disorders, depression, autism and attention deficit hyperactivity disorder (ADHD) (Rao *et al.*, 2008). The devastating situation of mental health problem in developing countries underscores the need for setting sustainable approach to address the issue. If the problem is left unattended, may lead to adverse socio and economic consequences at both individual and community levels.

2.2 Socio-economic consequences of mental health disorders

Mental disorders impose an enormous burden on society accounting for almost one in three years lived with disability globally (World Bank Group/ WHO, 2016). Additionally, mental disorders cause a significant economic burden due to lost economic output and the link between mental disorders and costly, potentially fatal conditions including cancer, cardiovascular disease, diabetes and obesity (Vigo *et al.*, 2016). The recent World Bank estimates show that the lost economic output caused by untreated mental disorders as a result of diminished productivity at work, reduced rates of labor participation, foregone tax receipts and increased welfare payments amounts to about US$1 trillion per year (World Bank Group/ WHO, 2016). More than 54% of the estimated global cost of mental disorders was borne by developing countries and by 2030 that cost is projected to reach 58%. These socio-economic impacts of mental health disorders underscore the need to reduce the prevailing burden of

mental health disorders at the household level and national level. The only sustainable method for reducing the burden caused by mental health disorders is prevention (WHO, 2014).

2.3 Poor nutrition as a major cause of mental health disorders

Poor nutrition has long been recognized as one the contributing factors of mental illnesses among individuals in developing countries (Korn, 2016). The most common nutritional deficiencies seen in patients with mental illnesses are omega-3 DHA and EPA fatty acids, B vitamins, minerals and amino acids, all of which are precursors to neurotransmitters (Rao *et al*., 2008). This implies that provision of products that are rich in omega-3 fatty acids, particularly the one with high biological value could address the problem of mental disorders in the affected population. Evidence from previous studies show that nutrient-based supplements might provide many neurochemical modulatory activities that are beneficial in the prevention and management of mental health disorders (Forsyth *et al.*, 2017). However, the most commonly used omega-3 products; especially in developing countries do not contain bioavailable omega-3 DHA. This underscore the need for looking a safe source of bioavailable omega-3 DHA and other essential nutrients that are direct linked to prevention of mental health illnesses.

2.4 Omega-3 based interventions for addressing mental health disorders

The field of nutrition has recently received attention as a sustainable solution the prevailing mental health disorders in both low and high income countries (Marx *et al.*, 2017; Ramakrishnan *et al.*, 2009). The assertion is based on clinical trials which have shown that nutrient-based supplements can provide many neurochemical modulatory activities that are beneficial in the prevention and management of mental health disorders (Forsyth *et al.*, 2017). Success stories of these clinical trials have prompted scientists from to develop are a number of nutrient-based interventions to promote mental health in the world (Panse & Phalke, 2016). Supplementation of omega-3 fatty acids, especially, docosahexaenoic acid (DHA) is an example of available nutrient-based interventions that have shown promising results in improving mental health. A number of studies have demonstrated usefulness omega-3 DHA in preventing and or treating mental health disorders (Sun *et al*., 2018; Thome Research Inc., 2009). Some of the reported mental disorders that have been treated using omega-3 DHA include but not limited to bipolar depression, major depressive disorder, post-

traumatic stress disorder, schizophrenia, psychosis and attention deficit hyperactivity disorder (ADHD).

Omega-3 DHA can provide a range of neurochemical activities via the following mechanisms: modulation of neurotransmitter (noradrenaline, dopamine and serotonin) re-uptake, degradation, synthesis and receptor binding; anti-inflammatory and antiapoptotic effects; and the enhancement of cell membrane fluidity and neurogenesis via upregulation of brain-derived neurotrophic factor (BDNF) (Sun *et al*., 2018). Some other nutrients that boost the activity of omega-3 DHA in the central nervous system include S-adenosyl methionine (SAMe), N-acetyl cysteine (NAC), zinc, B vitamins (including folic acid), magnesium, vitamin C and vitamin D (Sun *et al*., 2018; Thome Research Inc., 2009). This means that nutrient-based interventions that consider using sources or products that are rich in both the omega-3 DHA and its cofactors are likely to have a more significant impact on mental health improvement.

2.5 Prevailing sources of omega-3 DHA and their drawbacks

The DHA found in the brain is not produced *de novo* in mammals. Instead, it must be obtained from the diet or synthesized from the precursor fatty acid, alpha-linolenic acid (ALA) (Sun *et al*., 2018). Currently, fish oil is the major source of omega-3 DHA. However, several drawbacks related to the use of fish oil as the source of omega-3 have been reported in the literature (Lane *et al*., 2014). Fish oil possesses undesirable odors, flavors and tastes which discourage consumers from consuming it in its pure form. Furthermore, fish oils are likely to contain high levels of methyl-mercury, heavy metals and antibiotics, creating a risk of poisoning the consumers. In addition, environmental contaminants such as dioxins, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls have been found in fish oil, all of which discourage its use. Fish oil is also very expensive, thus, may not be afforded by majority of individuals in developing countries. In addition, the production of fish oil has reached the optimal threshold and is declining throughout the world. In this view, there is a need to look for sustainable and safe sources of omega-3 DHA.

Dietary ALA could be an alternative source of omega-3 DHA (Domenichiello *et al*., 2015). This is because human bodies can convert dietary ALA into omega-3 DHA and that the ALA-rich foods are almost found everywhere on this earth. Some of foods that are rich in ALA include walnuts, soybeans, flaxseeds, chia seeds, canola, and many other nuts and

seeds. Beans, legumes and wheat germ are also high in omega-3 ALA (Lenihan-Geels *et al.*, 2013). Several foods and food products that are rich in ALA are available on the global market and are being advertised as food for brain development and cognitive function in all age groups. People believe that since human bodies can make DHA out of ALA, eating more omega-3 ALA foods would help them get enough DHA. Unfortunately, the body is not very good at turning omega-3 ALA into omega-3 DHA (Lane *et al.*, 2014). The reasons to such inefficiency in ALA conversion are not clearly stated. However, some evidence show that poor levels of cofactors such as zinc, magnesium, vitamin C, proteins and B vitamins and too much omega 6 in the body slowdown the conversion rate of ALA into DHA (Saunders *et al*., 2012).

Although adequate levels of cofactors and the optimal ratio of omega-3 to omega-6 increase the conversion rate of ALA into DHA, the output of this conversion may not confer physiological benefits in the brain (Gillingham, 2013). Thus, individuals who solely depend on ALA-rich foods for their DHA are likely to be deficient in this important polyunsaturated fatty acid. The deficiency in DHA is very common among the individuals in developing countries, probably because they rely only on ALA-foods for their DHA. Evidence from the recent global estimates of dietary DHA shows that the vast majority of infants and young children in developing countries fall well short of the recommended intake (100 mg/day) of DHA omega-3 fatty acid (Forsyth *et al.*, 2016). This highlights the need for setting a robust strategy to improve the DHA status in resource-poor communities.

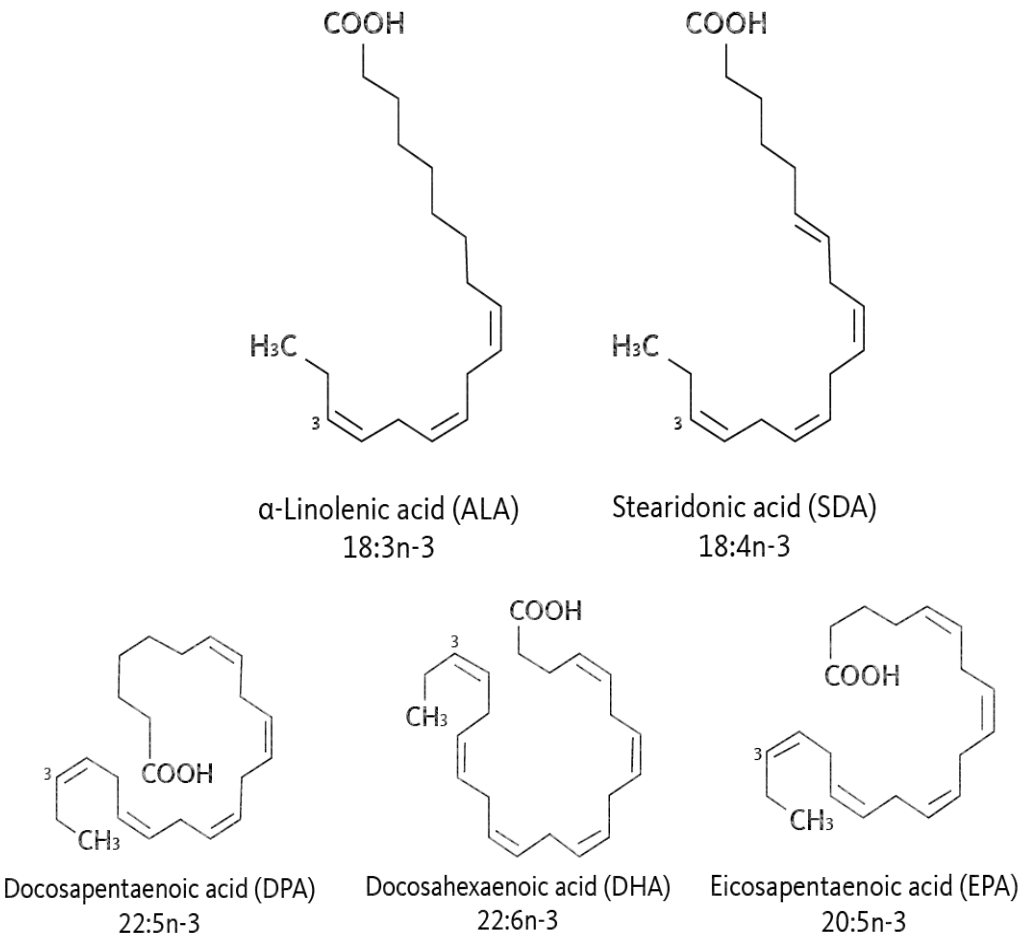
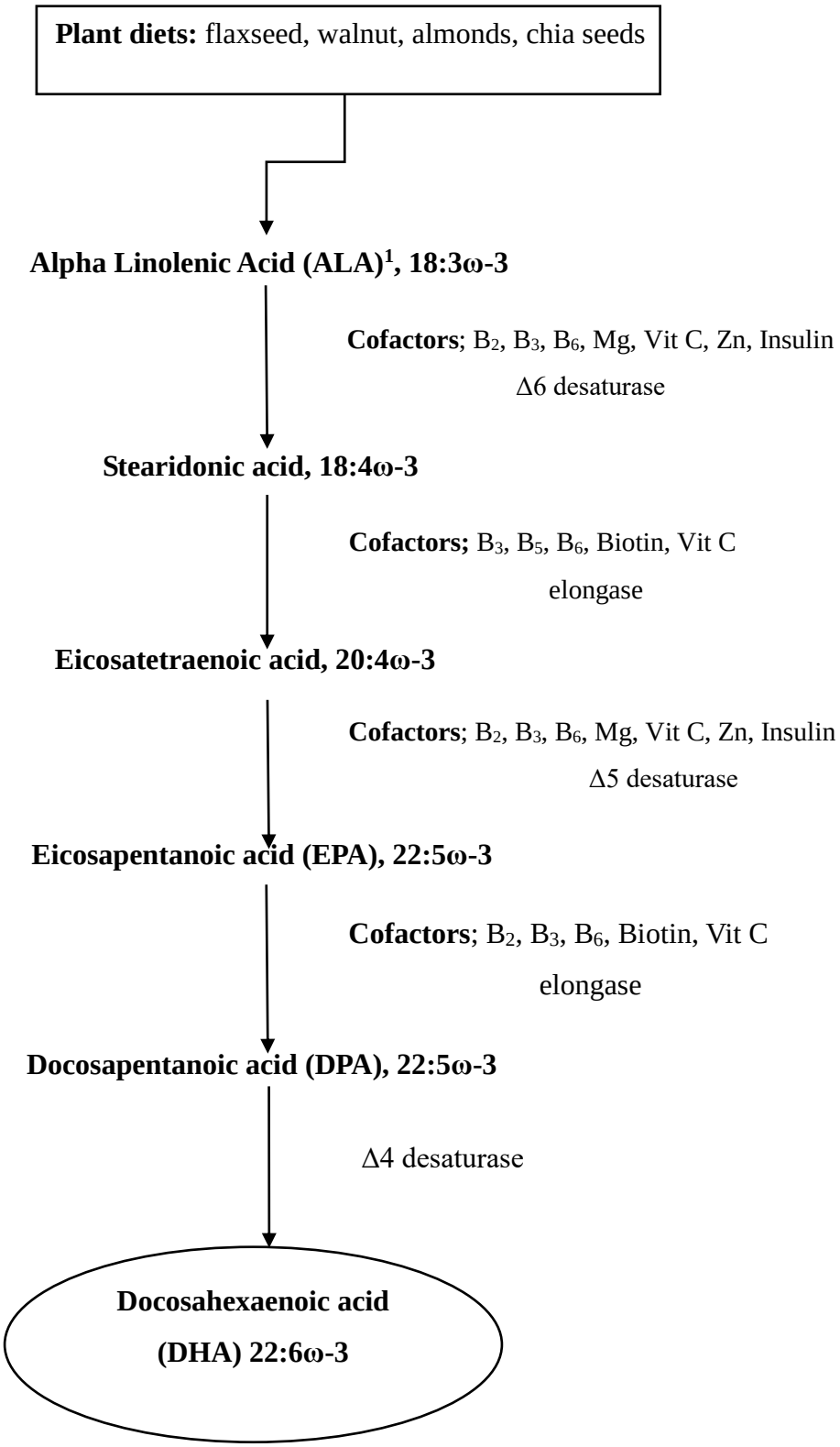


Figure 1: Molecular structures of dietary omega-3 fatty acids



[1]About 0-9% of ALA is converted to EPA and DHA. Source: (Lane *et al*., 2014)

Figure 2: Conversion of alpha linolenic acid (ALA) to docosahexaenoic acid (DHA)

2.6 Microalgae as an alternative source of bioavailable omega-3 DHA

Since the conversion of ALA into DHA is quite inefficient, involving many desaturase and elongase enzymes, many researchers speculate that there could be benefits in providing pre-formed DHA in the diet rather than relying solely on ALA. This is particularly challenging during fetal and neonatal development when demands for DHA in the central nervous system cannot readily be met by ALA alone (Sun *et al*., 2018). Researchers have further speculated that the primary source of food for fish could be the appropriate and sustainable source of dietary preformed DHA. This is because, even fish themselves cannot synthesize DHA *de novo* in their body (Dewick, 2009). They obtain their DHA from the foods they eat, normally phytoplankton (microalgae).

Microalgae is the best alternative natural source of bioavailable omega-3 DHA and other essential nutrients such as iron, zinc, vitamin B_3, vitamin B_6, vitamin C, vitamin E and magnesium, some of which are cofactors for the conversion of ALA into DHA in the body. Because of this, microalgae have recently been pointed out by 130 national academies of science and medicine (as reported by AIP, 2018) as one of the innovative foods that can bring co-benefits to human health and climate in the near future. Increased consumption of microalgae will replace meat consumption in some regions and hence reduce emission of greenhouse gases that emanates from the meat. So, microalgae have multiple benefits such as promotion of nutrition and health, generation of income to smallholder farmers, and mitigation of climate change in developing regions like East Africa.

2.7 Opportunities for future research around microalgae in East Africa

A number of microalgae species have been identified across the globe. The typical largest homes of microalgae are Japan, the Philippines, China and South Korea, followed by Vietnam, Chile, and Eastern Africa (Tanzania in particular) (Rajauria *et al.*, 2015). Unlike other countries, Eastern Africa has not yet fully exploited the potential of microalgae to improve nutrition and health in the region. So far, there is no scientific information regarding the nutritional and phytochemical profiles of microalgae found in the region. This limits practitioners from incorporating microalgae in nutrient-based interventions for improving status of omega-3 DHA among the individuals in the region. This stresses the need to identify microalgae species in the region and establish their scientific evidence in improving nutrition and health. This scientific information will help nutritionists and other health practitioners

develop formulations or products rich in preformed omega-3 DHA for prevention of mental health disorders in the region.

A proper business model that can link microalgae smallholder farmers and processors is also needed in the region. This model is very important, as it will accelerate optimal utilization of microalgae in the region. Optimization tools like linear programming (LP) can be deployed to ensure keen execution of the model along the microalgae chain. Linear Programming is a mathematical tool which allows generation of optimal solutions that satisfy prescribed constraints at once (Dibari *et al.*, 2012). The tool can be used to optimize production conditions for microalgae. It can also be used by microalgae processors to formulate products that are rich in preformed omega-3 DHA and other essential nutrients (Cahyaningrum *et al.*, 2016). The tool can also be used to inform regulatory authorities to design appropriate policies and dietary guidelines for omega-3 DHA that are currently missing in East Africa.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Formulation and testing of the product

The overall process of formulating and testing the model formula involved three key phases. The first phase encompassed the identification of the target group and the desired composition of the formulation. After that, global and local food composition databases and published reports were used to identify potential ingredients and nutrient composition data. The second phase involved the activity of defining decision variables, constraints and objective function for the linear programming model. These parameters were used to set up and solve the LP model using a widely available software (Microsoft Excel Office 2013 and the Solver add-in). The third phase involved preparation and sensory testing of the model formulations in the food kitchen at NM-AIST laboratory. In this phase, the prepared formulations were also assessed for the feasibility of mixing in the laboratory and predicted the feasibility of full-scale production.

3.1.1 Choice of food composition database

Optimal formulation of this study relied a lot on accuracy of the food composition database that were selected. The selection was based on criteria set forth in a previous study (Dibari *et al.*, 2012), which included;

(i) The widest representation of commodity composition data from around the world

(ii) The largest number of nutrient data values per food

(iii) Food descriptors matching the selected ingredients

(iv) Internationally respected datasets, ideally with methods cited in peer reviewed journals.

The key food composition database identified include; Tanzania Food Composition Tables, United States Department of Agriculture National Nutrient Database, Canadian Nutrient File, Danish Food Composition Database, and FAO/INFOODS Food Composition Databases.

3.1.2 Selection of potential ingredients

In the selection process, a checklist of all potential ingredients that can be sourced or farmed in Tanzania was prepared. Thereafter, a final selection based on the nutrient content, local availability and acceptability of the local ingredients was made. Care was taken to include ingredients rich in preformed omega-3 DHA, high quality protein and other essential nutrients like zinc, magnesium, niacin, vitamin B_6, vitamin C and vitamin E that favor conversion of ALA to DHA. Microalgae, golden berries, pumpkin seeds and sesame seeds are some of the key ingredients that were chosen as they are very rich in health promoting compounds, and can be sourced locally in Tanzania and other Eastern Africa countries. Microalgae for instance, are rich in preformed Omega-3 DHA and other essential nutrients. The nutritional information for each ingredient was collected from the following sources; the USDA Nutrient Database, Tanzania Food Composition Tables, Canadian Nutrient File, Danish Food Composition Database - Frida version 3 and SCI peer reviewed journals.

3.1.3 Setting up and running the LP model

Optimal formulation was designed to satisfy several pre-stated conditions (constraints), which were derived from UN specifications set for ready to use therapeutic foods. Some of these constraints include; energy and nutrient concentration, palatability, texture, total food ingredient weight, and the ratios of protein, fat and essential fatty acids to energy. The LP tool was set to consider minimization of cost as the objective function. The linear objective function for this study was expressed as follows;

$$Y = \sum_{i=1}^{n} b_i a_i B_i \quad (1)$$

where Y is the total cost of ingredients, bi is the cost per 1g of ingredients i, and Bi is the amount of ingredient i as described in the previous study (Brixi, 2018).

Energy and nutrient concentration constraints. The LP constraints were introduced to ensure that the formulation met the international standards for energy density and the ratios of protein, fat and essential fatty acids to energy. Care was taken to ensure that the energy density is equal to 500 kcal/100 g, and caloric distribution to be 45% to 50% from fat and

10% to 12% from protein as per international specifications (UNICEF, 2013). Furthermore, constraints were introduced to ensure that the optimal formulation contains an elevated level of preformed Omega-3 DHA+EPA and other essential nutrients. Protein digestibility corrected amino acid score (PDCAAS) was also calculated and constrained to ensure protein quality (Brixi, 2018). The PDCAAS was calculated using the following equations;

$$Qa = \sum_{i=1}^{n} C_i a_i d_i \quad (2)$$

$$\frac{Qa}{P} \geq 0.95\ (g_a) \quad (3)$$

where Qa is total quantity of essential amino acid a, Ci is quantity of ingredient i, ai is quantity of amino acid a per 1 g of ingredient i, di is protein digestibility factor of ingredient i, P is total quantity of protein in the formula, and ga is goal quantity of amino acid a per 1 g reference protein.

Palatability constraint was also introduced in the LP model to ensure acceptable tastes of the optimal formulation. In this design, dried golden berries were included in the formulation to serve a purpose of sweet taste. As for the texture related constraints, a paste-like property was considered as a suitable consistency and texture of the formulation. This is because paste texture property can be squeezed easily into the mouth by children or by their caregivers. Particle size and fat composition was constrained as they can significantly affect the texture quality of the formulae.

Once the formulation was shown to be acceptable (as described in a subsequent section on the preparation of prototype formulation), the total food ingredient weight was constrained to allow a sufficient space for inclusion of vitamin and mineral fortificant for severely malnourished children. In this exercise, an equality constraint was deployed to fix the weight of the formulation at 97-100 g. This setting is based on previous calculations which showed that up to 3% of the final product weight might be required for the premix nutrients (Ryan *et al.*, 2014).

The LP model and the software. The process of running the LP model consisted of: (a) creation of the data layout on a Microsoft Excel spreadsheet; (b) activation of add-in Solver

Function, which is supplied with standard installations of Excel; (c) assignment of the objective function (OF), decision variables and constraints; (d) running the LP procedure to solve the OF; and (e) a sensitivity analysis. Steps (a) to (c) were applied following the procedure described elsewhere (Dibari *et al.*, 2012).

3.1.4 Preparation of prototype formulations

Prototype formulations were prepared at the NM-AIST laboratory kitchen. The preparation underwent different steps, including; roasting of some ingredients, particle size reduction, homogeneous blending, and packaging. In the present exercise, qualitative questions, such as "can this formulation be mixed with a counter-top mixer? A food processor? A blender?" and "Is it possible for this formulation to be mixed in a bakery mixer? In a ribbon blender? Or other mixing equipment?" were answered for each formulation as described in a previous study (Ryan *et al.*, 2014). Anti-nutritional factors, anticipated ingredient interactions and sensory attributes were also assessed qualitatively. Based on these assessments, 10 formulations were prepared in the laboratory kitchen. Individuals familiar with ready to use therapeutic foods then tasted all 10 prepared formulations informally. Basic tastes were rated informally on a 9-point hedonic scale. The two most promising formulations were chosen and informally evaluated for sensory attributes by three independent experienced nutritionists who then proposed one novel formulation as an alternative to typical ready to use therapeutic foods in the developing world.

3.2 Laboratory analysis of the prototype formulation

3.2.1 Proximate composition analysis

Proximate analysis of the prototype was carried to determine moisture content, crude protein content, crude fat content, crude fiber content, ash content and carbohydrate content as described below;

(i) Moisture content

Moisture content was determined using a published standard method of Asean manual of food analysis of 2011. Sample was dried in an oven at controlled temperature to constant weight. Into a cleaned, dried and weighed glass crucible, 5 g of the dried sample was weighed. Then, the crucible with the content was further dried at 103 ℃ for 12 hours in the

oven. The sample had to be cooled in a desiccator and re-weighed. The weight loss (expressed as a percentage of the initial weight of the sample) gives the amount of moisture in the sample (equation 4).

$$\text{Moisture (g/100g)} = \frac{(W2 - W3)x100}{(W2 - W1)} \quad (4)$$

where: W1= weight of crucible (g)
W2= weight of crucible + sample before drying (g)
W2 - W1 = weight of sample (g)
W3 = weight of crucible + sample after drying (g)
W2 – W3 = loss of weight (g).

(ii) Crude protein content

Crude protein was determined by the method of the Association of Official Analytical Chemists (AOAC) as described by (Eshun *et al*., 2013). Briefly, 2 g of the sample was weighed into a digestion flask. 0.5 g of selenium catalyst was added, followed by 25 ml of conc H_2SO_4, then the flask was shaken to mix the contents. The contents were heated for 8 hours until the solution turned green and clear. The sample solution was then transferred into a 100 ml volumetric flask and made up to the mark with distilled water. Into a 250 ml conical flask, 25 ml of 2% boric acid was pipetted and two drops of mixed indicator (20 ml of bromocresol green and 4 ml of methyl red) solution were added; followed by the addition of 15 ml of 40% NaOH solution into the decomposition chamber of the distillation apparatus. Then, into a Kjedahl flask, 10 ml of the digested sample solution was introduced, and then the condenser tip of the distillation apparatus was dipped into the boric acid. Distillation of ammonia in the sample solution was done into the boric acid until it turned completely to bluish green. Titration of the distillate was done using 0.1 N HCl solution, until it turned colorless. A conversion factor of 6.25 was used to calculate the total percent of nitrogen and crude protein as shown in equation 6. Point to note is that in every batch of analysis, a blank test was included so as to subtract reagent nitrogen from the sample nitrogen.

$$\text{N (g\%)} = \frac{(\text{mL 0.1N HCl sample} - \text{mL 0.1N HCl blank})x\ 0.0014\ x\ \text{N HCl}\ x100}{(\text{Weight of sample})} \quad (5)$$

$$\text{Crude protein (g/100 g)} = (\%\ \text{total nitrogen}) \text{x nitrogen conversion factor (6.25)} \quad (6)$$

(iii) Crude fat content

Crude fat was determined based on the Sohxlet extraction method of Association of Official Analytical Chemistry described in the Asean manual of food analysis of 2011. Briefly, a 250 mL quick fit round bottom flask was washed and dried in an oven at 103 ℃ for 25 minutes, left to cool to room temperature and then weighed. Into a muslin thimble, 2 g of the sample was weighed, and the thimble was inserted into the extraction column where the condenser is connected. Thereafter, into the round bottomed flask, 200 ml of the extracting solvent (petroleum ether, boiling point 40 to 60 ℃) was poured and fitted into the extraction unit. With the aid of electro thermal heater, the flask was heated at 60 ℃ for 2 hours.

The condenser was used to cool and reflux the evaporated solvent due to heating. After the extraction process, the thimble was removed and the solvent was recovered by distillation. To evaporate the solvent, the flask containing fat and the residual solvent was placed on a water bath and further dried in an oven at 103 ℃ for 30 minutes to entirely remove the solvent. The flask was then cooled in a desiccator and re-weighed. The amount of fat obtained was expressed as a percentage of the initial weight of the sample as described in equation 7.

$$\text{Total fat (g/100g)} = \frac{(W3 - W2)\ x\ 100}{W1} \quad (7)$$

where: W1= weight of sample
W2= weight of dried flask before fat extraction
W3 = weight of dried flask after fat extraction

(iv) Crude fiber content

Fiber was determined by the method of Association of Official Analytical Chemists (AOAC) described in a previous study with little modification (Eshun *et al.*, 2013). The defatted sample (from crude fat determination) was transferred into a 750 ml Erlenmeyer flask and 0.5 g of asbestos was added. Thereafter, 200 ml of boiling 1.25% s H_2SO_4 was added and the flask was immediately set on a hot plate and condenser connected to it. The content was brought to boil within 1 minute and the sample was digested for 30 minutes.

At the end of 30 minutes, the flask was removed and the content was filtered through a linen cloth in a funnel and subsequently washed with boiling water until the washings were no longer acidic. The sample was washed back into the flask with 200 ml boiling 1.25% sodium hydroxide solution. The condenser was again connected to the flask and the content of the flask was boiled for 30 minutes. It was then filtered through the linen cloth and thoroughly washed with boiling water until the washings were no longer alkaline. The residue was transferred to a clean crucible with a spatula and the remaining particles washed off with 15 ml ethanol into the crucible.

The crucible with its content was then dried in an oven overnight and cooled in a desiccator and weighed. The crucible with its content was then ignited in a furnace at 600 ℃ for 30 minutes, cooled and reweighed. The loss in weight gave the crude fiber content and was expressed as a percentage of the initial weight of the sample.

(v) Ash content

Ash was determined by the method of Association of Official Analytical Chemists as (AOAC) described in the Asean manual of food analysis of 2011. Briefly, about 2.0 g of sample was weighed into a dried and weighed porcelain crucible. The crucible with its content was placed in a furnace preheated to 600 ℃ for 2 hours. The sample was allowed to cool in the furnace to 250 ℃. The crucible and the ash were then transferred into an oven at 100 ℃ for 30 minutes cooling. After this period, the crucible with its content was cooled in a desiccator. The crucible with its content was weighed. The weight of the ash was expressed as a percentage of the initial weight of the sample.

$$\text{Ash g per 100 g} = \frac{(W3 - W1)\ x\ 100}{(W2 - W1)} \qquad (8)$$

where: W1= weight of crucible
W2= weight of crucible + sample
W3= weight of crucible + ash

(vi) Carbohydrate content

Total percentage carbohydrate was determined based on the method reported previously (Eshun *et al*., 2013). In this method, carbohydrate content was determined by subtracting the

total values of protein, fat, fiber, moisture and ash constituents of the sample from 100 as expressed in equation 9 below.

$$\text{Carbohydrate} = 100 - (\%\ \text{moisture} + \%\ \text{ash} + \%\ \text{crude protein} + \%\ \text{crude fat} + \%\ \text{crude fibre}) \quad (9)$$

3.2.2 Mineral analysis

Analysis of minerals was done according to the previous method (Altun *et al*., 2017). Briefly, 100 g of sample were dissolved in nitric acid for acid digestion and ultra-pure water was added to the solution until the volume reached 100 ml. Then after, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to profile the mineral composition of the sample. The glass-ware was cleaned with % 10 (v/v) HNO_3 solution for one day and rinsed with ultrapure water. About 1.0 g of the sample was digested with 4.0 mL of 65% (v/v) HNO_3 and 0.5 mL of 35% (v/v) H_2O_2 in PTFE vessels. The vessels were placed into microwave system. A blank digest was carried out in the same way. Digestion conditions for the microwave system applied were as follows: up to 120 ℃ for 15 minutes and then constant for 10 minutes; up to 160 ℃ in 20 minutes and constant for 15 minutes; finally, a cooling stage (30 minutes) was carried out to 22 ℃ and diluted to 50 mL with deionized ultrapure water. This solution was finally used for minerals analysis, performed with an ICP-MS. The concentrations of nine elements (sodium, potassium, calcium, phosphorus, magnesium, iron, zinc, copper and selenium) were then determined in the sample. The digested sample was analyzed in duplicate and measured in triplicate by ICP-MS detection. All reagents were of analytical grade.

3.2.3 Water soluble vitamins analysis

Analysis of water soluble vitamins (B vitamins and vitamin C) was done as follows:

(i) B vitamins

Extraction for vitamins analysis was done according to a method described previously (Sami *et al*., 2014). Briefly, about 2 g of the sample was placed in 25 mL of H_2SO_4 (0.1 N) solution and incubated for 30 minutes at 121 ^oC. The contents were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, and 50 mg Takadiastase enzyme was added. The preparation was

stored at 35ºC overnight. The mixture was then filtered through a Whatman No.4 filter, and the filtrate was diluted with 50 mL of pure water and filtered again through a micropore filter (0.45 µm). Twenty microliters of the filtrate were injected into the HPLC system. Quantification of vitamin B content was accomplished by comparison to vitamin B standards. Standard stock solutions for thiamine, riboflavin, niacin, vitamin B_6, vitamin B_{12}, Folate, pantothenic acid and choline were prepared according to standard methods reported previously. Chromatographic separation was achieved on a reversed phase- (RP-) HPLC column through the isocratic delivery mobile phase at a flow rate of 0.5 mL/minute. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

(ii) Vitamin C

For vitamin C, 10 g of the sample was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M) as reported by Sami *et al*. (2014). The mixture was placed in a conical flask and agitated at 10 000 rpm for 15 minutes. The mixture was then filtered through a Whatman No.4 filter, and samples were extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (0.4 M) solution at a final concentration of 0.1 mg/Ml. The calibration line was converted to a linear range based on four measured concentration levels. Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase at a flow rate of 1mL/minute. UV absorbance was recorded at 254 nm at room temperature.

3.2.4 Fat soluble vitamins analysis

Analysis of fat soluble vitamins were done according to the previous method (Sami *et al*., 2014). Briefly, in 10 g of the sample, 1 g of pyrogallic acid, 70 mL ethanol and 30 mL (50%) KOH were added, stirred, and refluxed for 40 minutes using a water bath at 50 ± 2 ℃. Extracts were obtained three times using various ether concentrations (50 mL, 30 mL and 20 mL). Double-distilled water was used to neutralize the extract, which was dehydrated using anhydrous sodium sulfate. Further, the extract was concentrated to approximately 5 mL by using a water bath (50 ± 2 ℃), diluted to 10 mL by using methanol, filtered using a 0.45 μm membrane, and finally subjected to HPLC analysis.

The HPLC analysis was performed with the Agilent 1100 series HPLC system (Agilent; USA), including a diode array detector. The column was made of stainless steel. The Agilent Eclipse XDB-C18 column was used (5 μm, 4. 6 × 150 mm), and the solvent was methanol. The UV detection was recorded at 325 nm for vitamin A, 290 for vitamin E and 244 nm for vitamin K. Separation of all vitamins was based on isocratic elution and the solvent flow rate was maintained at 1 Ml/minute. About 20 microliters of the sample oil were directly injected into the HPLC column. Fat-soluble vitamins were identified by comparing their retention times with those authentic standards. All procedures were carried out under subdued light conditions.

Standard solutions of vitamins were prepared by serial dilution to concentrations of 1, 2, and 5 mg per liter of vitamins E, K and A, respectively. Standard solutions were prepared daily from a stock solution, which was stored in the dark at -20 ℃. Thereafter, 20 microliters of standard solution were injected, and peak areas were determined to generate standard curves.

3.2.5 Fatty acids analysis

Analysis of fatty acids was based on a previously published method (Kchech, 2017). About 150 mg of the sample were dissolved in 0.5 N methanolic sodium hydroxide and heated on a steam bath. Then after, 5 ml of BF3 methanol were added to the solution and the mixture was boiled. To float the methyl esters up, enough of saturated sodium chloride was added to the solution. The solution was left to cool at room temperature and distilled water was added followed by the addition of n-hexane. The solution was then transferred into a separating funnel and left to settle until a clear separation between the organic phase and the aqueous phase was visible. The aqueous layer was drained first into a conical flask. The organic layer was transferred to the second conical flask followed by the addition of anhydrous sodium sulfate to remove any trace of water in the organic layer. The methylated fatty acids contained in n-hexane was then transferred to vials and nitrogen gas was blown to remove hexane.

The vials were placed on a liquid auto-sampler and a 1.0 µL injection of the prepared sample was introduced into the GC using 10.0 µL Agilent syringe. The syringe was washed prior to each injection with one portion of ethyl acetate, and one portion of dichloromethane. The injection was introduced into an Agilent single tapered splitless liner containing glass wool at 240 ℃. The GC separation was accomplished using an Agilent Technologies 6890 N

Network GC System containing an Agilent J & W GC column, DB-5MS; with a length of 30 m, diameter of 0.250 mm and film of 0.25 µm. The carrier gas was helium with a constant flow of 1.4 mL/minute. The initial oven temperature was held at 45 ℃ for 5 minutes, increased at 10 ℃ for a final temperature of 320 ℃ and held at 5 minutes. The transfer of the sample to the detector was done through a transfer line that was kept at 300 ℃. The detector used for performing the analysis was mass spectrometer that had a relative electron impact ionization (EVI) at 70 eV, source temperature of 230 ℃ and a quadrupole temperature of 150 ℃. The total runtime was 37.5 minutes with an initial solvent delay of 6.50 minutes with a scan range from m/z 60-600 at a normal scan speed with a rate of 2.71 scans per second. The concentration of methylated fatty acids in the sample was calculated based on the normalized peak area and the equations obtained by the calibration curves.

3.2.6 Amino acids analysis

Amino acid profile was determined using HPLC-florescent detection method (Dayal *et al*., 2011). Briefly, the amino acid composition was analyzed after sealed tube hydrolysis with 6 N HCl for 22 hours at 110 ℃. After the hydrolysis, the acid was evaporated in a vacuum oven and the sample was kept in NaOH dessicator to remove traces of acid. The residue was brought in to 1 ml of Sodium Citrate-Perchloric acid sample diluent (pH 2.20) and filtered through 0.2 µm membrane filter. Separation of amino acids was done in a column packed with a strongly acidic Na+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elusion at a flow rate of 0.3 ml/minute by using two buffers, (A) sodium citrate-perchoric acid (pH 3.2); (B) boric acid sodium citrate-sodium hydroxide (pH 10.0). The amino acids were quantified using a fluorescent detector after post column derivitization with O-pthaladehyde and 2-mercaptoethanol. Amino acid standard solution for fluorescent detection was used as external standard.

3.3 Relative difference of the LP calculated values and the Lab analyzed values

The relative difference of the LP calculated values and the lab analyzed values was calculated based on the method described by Dibari *et al*. (2012). In this method an absolute difference for each nutrient was calculated by subtracting the LP calculated values from lab analyzed values. Thereafter, the relative difference was calculated by dividing the absolute difference values over the LP calculated values as shown in equation 10. The relative difference was expressed as percentage.

$$\text{Relative difference} = \frac{(C)x100}{A} \quad (10)$$

where: A= LP calculated values

B= Lab analyzed values

C= Absolute values (B-A).

3.4 Sensory evaluation of the final product

Sensory evaluation test was conducted to determine the degree of consumer acceptability of the formulated ready-to-use food. The hedonic test was conducted among mothers or primary caregivers. The assumption was that mothers or primary caregivers are more likely to decide on what a child should eat at household level. Inclusion criterion was that a mother or primary caregiver must have a child aged between six months and 10 years and is willing to participate in the experiment. The number of respondents who turned up for this experiment was 50. The acceptance of sensory attributes of the formulated food product was evaluated using a nine-point hedonic scale as described by Megido *et al*. (2016). The scale ranges from "extremely dislike" to "extremely like", where 1= like extremely, 2= like very much, 3 = like moderately, 4=like slightly, 5=neither like nor dislike, 6= dislike slightly, 7= dislike moderately, 8= dislike very much, 9= dislike extremely with respect to color, odor, taste and texture. Respondents were instructed orally to neutralize any taste (drink water) before the actual sensory evaluation test. Thereafter, each respondent was given a product for hedonic test. Their responses were recorded and coded for statistical analysis.

3.5 Assessment of willingness to pay (WTP)

The WTP was elicited using Becker-DeGroot-Marschak (BDM) method as described in previous studies (Adams *et al*., 2016; Lybbert *et al*., 2016). Briefly, primary caregivers were asked if they are willing to pay specific, incrementally-increasing prices for the modelled ready-to-use formula (100 g) to feed their children. Once a participant indicates, s/he is not willing to pay a specific price, his/her maximum WTP was recorded as the previous price in the series. The auctions were grouped in 10 sessions. Each session comprising somewhere between four to six participants. In each session, participants were asked to confidentially bid the price they are willing to pay for 100 g of ready-to-use formula to their assigned enumerator. The session was managed by the same facilitator to ensure similarities of the experiment as possible. During the auctions, the facilitator provided the nutritional and

health benefits of the product so that caregivers can provide informed decision regarding their WTP. The information that was provided to each session was the potential short-term benefits as well as long-term benefits of omega-3 DHA and other essential nutrients on brain development and cognitive function of a child, focusing on schooling attainment and economic productivity in adulthood. The experiment of WTP was done immediately after the sensory evaluation of the product.

3.6 Statistical analysis

Paired sample t-test was used to compare the average nutritional values between the LP calculated values and the laboratory analyzed values. The aim of this statistical test was to check whether the nutritional values of the formulated product and those from LP were statistically different at p=0.05. on the other hand, a Kruskal-Wallis H test was used to determine if there are statistically significant differences between sensory attributes (such as color, odor, taste and texture) of the product on a nine-point hedonic likert scale scores at p=0.05. Furthermore, in order to understand the determinant of WTP in the sample population, generalized linear model (GLM) was used to estimate the bid price while controlling for potential confounding factors. In the analysis, socio-economic factors (such as education, household size, annual income and occupation) and sensory attributes scores with respect to color, odor, taste and texture of the product were incorporated. Data were processed and coded in IBM SPSS version 21. All analyses and plots were carried out in SPSS and R-software version 3.2.5 (R Development Core Team, 2015).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Attributes of the final product

Optimal RUF formulae rich in preformed omega-3 DHA and EPA was developed using linear programming (LP) method. The formulation was based on ingredients that can be sourced locally in Tanzania and East Africa at large. Microalgae powder, dried fruits and roasted oily seeds were the common ingredients the LP tool selected to generate the optimal RUF formulae. Most of macronutrient and micronutrient contents in the proposed RUF were similar to the standard RUF (Table 1). For example, protein-energy ratio (0.19), fat-energy ratio (0.55), (n-3) fatty acids-energy ratio (0.003), (n-6) fatty acids-energy ratio (0.03) and the total energy content (524 kcal) of the optimized RUF formulae matched the UN standards.

The optimized RUF formulae contained high concentration (220 mg/100 g) of preformed omega-3 DHA and EPA. Similarly, the optimized RUF formulae contained a significant amount of natural cofactors required for the conversion of omega-3 ALA into omega-3 DHA in the body. In addition, the optimized RUF formulae contained a relatively well-balanced omega-6 to omega-3 fatty acids (2.5:1) ratio compared to the commonly used RUFs. The optimized formulae also achieved the UN standards for phytic acid to iron ratio, phytic acid to zinc ratio, calcium to phosphorus ratio and zinc to copper ratio which are determinants of mineral absorption in the body. In view of protein quality, the optimized RUF formulae achieved more than 95% protein digestibility corrected amino acid score (PDCAAS) (Table 1).

Likewise, the total cost of the proposed optimized RUF formulae was more than 50% lower than the estimated ingredient cost of the currently used Plumpy'nut formulae. Ingredient cost reduction in the optimized RUF formulae is largely driven by the replacement of milk powder and peanuts. The main cost savings generated in the optimized RUF formulae for Tanzania stem from the removal of milk powder, as well as from the low local price of local ingredients. The cost for main ingredient in the optmised formulae was about USD 0.04 for microalgae powder and dried fruits, USD 0.05 for sesame seeds and USD 0.06 for pumpkin seeds, all of which are locally available in the country**.**

4.1.2 Relative difference between LP calculated values and lab analyzed values

Paired sample t-test analysis showed that there was no statistical significance difference (p=0.102) between the LP calculated nutrient values and the laboratory analyzed nutritional values of the formulated product. The relative differences in the nutritional values between the LP calculated values and lab analyzed values are described in Table 2.

4.1.3 Characteristics of respondents participated in the study

Sixty primary caregivers participated in the sensory evaluation and willingness to pay experiments for the formulated ready-to-use food for children. All caregivers had children aged between 6 months and 10 years. Most common caregivers were mothers with a mean age of 28 years. About half of the studied households had more than five members and 33.5% of children had another sibling under 5 years of age. About half of primary caregivers had formal primary education, whereas more than one-third (35%) of the caregivers had no education at all. Most of primary caregivers were peasants engaged in subsistence crop farming. Table 4 summarizes the characteristics of the respondents who participated in the sensory evaluation and WTP experiment.

4.1.4 Sensory evaluation and willingness to pay results

For sensory testing, a Kruskal-Wallis H test showed that there was no statistically significant difference likert scale score between the sensory attributes of the ready-to-use product, with a mean likert scale score of 2.15 for color, 2.24 for taste, 2.31 odor and 2.14 for texture at p=0.05. Descriptive analysis showed that about 80%, 81%, 84% and 80% liked the product very much for color, taste, odor and texture, respectively. The average score for overall acceptability of the formulation was approximately 2.0 (“like very much”).

For WTP, on average, individuals were willing to pay at least US$ 0.15 for the formulated ready-to-use food. The generalized linear model (GLM) analysis showed that annual income and product sensory attributes were not able to predict the WTP at the 5% level (Table 5). In contrast, the GML model results showed that caregiver’s education status was the most significant predictor of WTP for the product. The bid price increased with education, individuals with a relative higher education bided higher price than respondents with lower education levels.

Table 1: Nutrient composition of the proposed optimized RUF formulae (per serving)

Component	RUF optimized (100 g)	Plumpy'nut[1] (92 g)	UN Standards (100 g)
Moisture, g	3.4	2.9	<8
Energy, kcal	524	544	520–560
Protein, g	26	16.6	14.5–19.0
Fat, g	32	36	26.8–36.3
Ash, g	4.0	3.8	0.3–4.0
Carbohydrate, g	33	40.7	41–58
Added sugar, g	—	20.5	≤25
Fiber, g	9.9	4.8	<5
Sodium, mg	226	52.2	<290
Potassium, mg	1080	1070	1100–1400
Calcium, mg	315	399	300–600
Phosphorus, mg	592	493	300–600
Magnesium, mg	304	119	80–140
Iron, mg	15	31.6	20–25
Zinc, mg	7.0	19.9	18–23
Copper, mg	1.5	1.5	1.4–1.8
Selenium	20	25	20–40
Vitamin A, mg RE	0.92	1.16	0.8–1.2
Vitamin E, mg	3.0	39	0.2
Vitamin K, mg	22	12	15–30
Thiamin, mg	0.8	1.12	>0.5
Riboflavin, mg	0.9	1.97	>1.6
Vitamin C, mg	22	306	290–360
Vitamin B-6, mg	0.35	0.93	>0.6
Vitamin B-12, mg	12.7	2.6	>1.6
Folate, mg	63	200	0.2
Niacin, mg	6.0	7.94	0.5
Pantothenic acid, mg	0.96	4.73	0.3
Choline, mg	31	70	—
n–3 Fatty acids, % TE	0.3	0.18	0.3–2.5
n–6 Fatty acids, % TE	3.0	3.91	3.0–10.0
n–3 (DHA+EPA), mg	220	–	–
SFAs, g	5.0	15.5	—
MUFAs, g	9.8	12.6	—
PUFAs, g	12.2	4.09	—
trans Fat, g	0.05	0.18	<3% total fat
Phytic acid, g	0.44	0.333	—
Molar ratio			
Phytic acid: iron	0.003	0.89	<2.5
Phytic acid: zinc	0.006	1.66	<15
Ascorbic acid: iron	0.46	3.08	<3.8
Weight ratio			
Ascorbic acid: iron	2.0	9.68	3.0–16.0
Calcium: phosphorus	1.0	0.81	1.0–1.5
Zinc: copper	5.0	13.27	5.0–20.0
Zinc: iron	0.5	0.63	0.8–3.5
PDCAA, %	100	≥95	—
Total ingredient price (USD)	0.15	0.30	—

[1] Data for peanut paste-based ready-to-use food were obtained from previous reports (Bahwere *et al.*, 2017; Brixi, 2018)

Table 2: Relative difference of the LP calculated values and the Laboratory analyzed values

Component	LP calculated values	Lab analyzed values	[1]A.D	[2]R.D
	A	B	B-A=C	(C/A) x100
Moisture, g	3.4	3.5	0.10	2.94
Energy, kcal	524	523	-1.00	-0.19
Protein, g	26	25	-1.00	-3.85
Fat, g	32	33	1.00	3.13
Ash, g	4.0	4.1	0.10	2.50
Carbohydrate, g	33	32	-1.00	-3.03
Added sugar, g	—	—	—	—
Fiber, g	9.9	10	0.10	1.01
Sodium, mg	226	226	0.00	0.00
Potassium, mg	1080	1080	0.00	0.00
Calcium, mg	315	312	-3.00	-0.95
Phosphorus, mg	592	590	-2.00	-0.34
Magnesium, mg	304	304	0.00	0.00
Iron, mg	15	15.5	0.50	3.33
Zinc, mg	7.0	7.2	0.20	2.86
Copper, mg	1.5	1.55	0.05	3.33
Selenium	20	20.5	0.50	2.50
Vitamin A, mg RE	0.92	0.95	0.03	3.26
Vitamin E, mg	3.0	3.1	0.10	3.33
Vitamin K, mg	22	21	-1.00	-4.55
Thiamin, mg	0.8	0.83	0.03	3.75
Riboflavin, mg	0.9	0.94	0.04	4.44
Vitamin C, mg	22	22.8	0.80	3.64
Vitamin B-6, mg	0.35	0.36	0.01	2.86
Vitamin B-12, mg	12.7	13	0.30	2.36
Folate, mg	63	61	-2.00	-3.17
Niacin, mg	6.0	6.2	0.20	3.33
Pantothenic acid, mg	0.96	1	0.04	4.17
Choline, mg	31	30	-1.00	-3.23
n–3 Fatty acids, % TE	0.3	0.3	0.00	0.00
n–6 Fatty acids, % TE	3.0	3	0.00	0.00
n–3 (DHA+EPA), mg	220	222	2.00	0.91
SFAs, g	5.0	5.1	0.10	2.00
MUFAs, g	9.8	10	0.20	2.04
PUFAs, g	12.2	12.3	0.10	0.82
Trans Fat, g	0.05	0.051	0.00	2.00
Phytic acid, g	0.44	0.45	0.01	2.27
Molar ratio				
Phytic acid: iron	0.003	0.003	0.00	0.00
Phytic acid: zinc	0.006	0.006	0.00	0.00
Ascorbic acid: iron	0.46	0.46	0.00	0.00
Weight ratio				
Ascorbic acid: iron	2.0	2	0.00	0.00
Calcium: phosphorus	1.0	1	0.00	0.00
Zinc: copper	5.0	5	0.00	0.00
Zinc: iron	0.5	0.5	0.00	0.00
PDCAA, %	100	98	-2.00	-2.00

[1]AB; absolute difference

[2]RD; relative difference

Table 3: Amino acid profile of the study RUF obtained by laboratory analysis

Amino acid	RUF optimized, g/100 g	Plumpy'nut[1], g/100 g
Cystine	0.27	0.18
Methionine	0.46	0.25
Aspartic acid	1.90	1.39
Threonine	0.70	0.54
Serine	1.09	0.82
Glutamic acid	4.10	3.01
Glycine	1.24	0.53
Alanine	0.99	0.52
Valine	1.05	0.71
Isoleucine	0.84	0.60
Leucine	1.56	1.20
Tyrosine	0.74	0.56
Phenylalanine	1.11	0.72
Lysine	0.77	0.93
Histidine	0.53	0.37
Arginine	3.34	1.01
Proline	0.87	1.07
Tryptophan	0.40	0.20

[1]Data for peanut paste-based ready-to-use food were obtained from previous report (Bahwere *et al*., 2016)

Table 4: Socio-demographic characteristics of respondents

Variable	Variable description	Number of caregivers/ Frequency (%)	Min, Max
Age (years)	Age of the respondent in years (Mean ±SD)	28±6	16,55
Head of household	Proportion of respondents who are head of households	6 (10%)	
Household size	Number of people living in the household (Mean ±SD)	5±2.1	2,12
Marital Status	Married	49 (82%)	
	Single	5 (9%)	
	Widowed	2 (3%)	
	Separated	4 (6%)	
Caregivers education	Illiterate	21 (35%)	
	Primary	30 (50%)	
	Secondary	7 (12%)	
	Tertiary	2 (3%)	

Table 5: Generalized Linear Model (GLM) for the WTP of the food product

Variable	Estimate	95% CI	*P*-value
Intercept	2.01E+03	1243 to 2779	< 0.05
Annual income	2.95E-05	-3.0E-05 to 9.0E-05	0.35
Color of the product	2.15E+02	-167 to 597	0.28
Taste of the product	-4.74E+01	-387 to 293	0.76
Odor of the product	-4.46E+01	-424 to 334	0.83
Texture of the product	-1.89E+01	-230 to 192	0.84
Age of respondent	-1.90E+01	-42 to 4	0.12
Education of the respondent	1.53E+02	17 to 289	0.04
Household size	-1.49E+01	-85 to 56	0.66
Occupation of the respondent	-1.28E+01	-69 to 44	0.63

4.2 Discussion

This study has shown that local ingredients other than fish oil (if optimized) can ensure dietary adequacy of bioavailable omega-3 DHA and other health promoting compounds among individuals in East Africa. In the present study, linear programming was used to formulate an alternative ready to use therapeutic foods based on locally available and culturally acceptable ingredients rich in omega-3 and other essential nutrients. The LP analysis indicated that it is technically feasible to design an internationally acceptable formulation rich in preformed omega-3 DHA and other health promoting compounds. The key ingredients in the formulations were pumpkin seeds, sesame seeds, golden berries and microalgae, all of which can be farmed locally by smallholder farmers in Tanzania. The ingredients were processed and rationed in a way that the formulation favors bioavailability of most limiting essential nutrients and bioactive health promoting compounds. For example, all oilseeds were roasted prior to the grinding into paste, as the use of raw non-roasted ingredients could lead to the presence of potentially high levels of anti-nutritional factors and phytates (Agume *et al*., 2017). The ingredients were then rationed in a way that a consumer should get adequate amount of preformed omega-3 DHA and EPA from a single serving of the formulation. The formulation was also designed to favor dietary adequacy of vitamin B_3, vitamin B_6, vitamin C, vitamin E, magnesium and zinc, which are cofactors for the conversion of ALA into DHA in the body. So, consumers will have an advantage of getting preformed DHA direct from the formulation and the DHA from ALA conversion in the body. Consumers will also have the advantage of getting (from the formulation) high potent lysine, threonine, methionine, tryptophan, vitamin A and iron which are the most limiting nutrients in developing world (Bailey *et al*., 2015; FAO, 2013).

The analysis of this study has further shown that one serving of the present prototype formulation can provide about 220 mg of omega-3 DHA and EPA. This amount is enough to meet a daily recommended intake of omega-3 DHA and EPA for children aged between 2-10 years (FAO, 2010), the very age group that needs DHA and EPA for brain development and mental health. Also, the analysis showed that the present formulation is far better than the most commonly used ready to use therapeutic foods, such as Plumpy'nut and Plumpy'doz when it comes to composition of essential compounds (such as DHA and EPA) for mental health and cognitive development. Most of commonly used ready to use foods that are imported to Africa contains negligible or no preformed omega-3 DHA and EPA (Brixi,

2018). This means that the present formulation from locally available ingredients can replace Plump'nut when it comes to nutrients and bioactive compounds that are needed for brain development and mental health. Furthermore, the balance of omega-6 and omega-3 fatty acids (2.5:1) in the present formulation is within the ratio that can support development of neural tissues (Yehuda, 2003). This ratio further makes the present formulation superior over most commonly used ready to use therapeutic foods, which in most cases, their omega 6 to omega 3 ratios are not within the recommended balance. Basically, an ideal ratio of omega 6 to omega 3 ratio for most people is 4:1 – that's 4 omega-6s for every 1 omega-3 (Yehuda, 2003). Conversely, nutritionists and anti-aging experts suggest that the ratio should go even further, maintaining a 1:1 ratio or higher in favor of omega 3s. Unlike commonly used ready to use foods, the present formulation contains essential fatty acids from non-animal sources. Microalgae is a major source of preformed omega-3 fatty acids in the present formulation. This makes the present formulation a future promising product for all consumers including vegetarians, especially in areas with high episode of mental health illnesses. Microalgae is an excellent natural source of highly potent omega-3 fatty acids and other functional compounds that can benefit the brain health across the entire lifespan (Sathasivam *et al*., 2017; Wells *et al*., 2017). Because of its high content of functional compounds with high biological value, the World Health Organization (WHO) has regarded microalgae as a future human superfood, especially for the developing world. The present innovation takes advantage of Tanzania being one of the best countries with natural conditions that favor growth of this natural superfood (microalgae) in Eastern Africa region (Antonio, 2012).

Apart from omega-3 fatty acids, single serving of the optimized formulation contains 15 mg and 95 mcg of iron and vitamin A, respectively, which are reported as one of the most limiting nutrients among children in the developing world. This amount can achieve about 100% and 50% of recommended daily intake (RDI) for iron and vitamin A, respectively, for a 1 to 3-year-old child. This amount can also achieve about 60% RDI for iron and approximately 30% RDI for vitamin A, for an adolescent girl (FAO/WHO, 2004). Likewise, one serving of the optimized formulation can achieve 100% RDI for vitamin B_3, 100% RDI for vitamin B_6, 70% RDI for vitamin C, 60% RDI for vitamin E, 100% RDI for zinc and more than 100% RDI for magnesium for the child aged 12-36 months (FAO/WHO, 2004). All these nutrients are cofactors for the conversion of ALA into DHA in the human body. This means that the proposed optimized formulation is in a position to offer adequate amount of precursors which when absorbed can help consumers get more DHA from ALA

conversion in the body (Thome Research Inc., 2009). The formulation can also provide more than 100% RDI for lysine, threonine, methionine and tryptophan (limiting essential amino acids), respectively, for children aged between 1 and 2 years (FAO/WHO/UNU, 2007). In fact, the optimized formulation meets the recommended protein content of 10 to 12 percent of kcal and have a protein digestibility corrected amino acid score (PDCAAS) of 1.0 as per international standards (USDA, 2012). This makes the present formulation to have the protein with high biological value as that of meat and fish. The energy content (520 kcal) of the present formulation (Table 1) meets the international standards for ready to use therapeutic foods, which ranges between 520-550 kilocalories (kcal) per 100 g. The contribution of fatty acids to this energy is 0.3% for total omega-3 fatty acids and 3% for total omega-6 fatty acids, all of which are within the international specifications for ready-to-use foods (USDA, 2012). Likewise, the present formulation does not contain artificial antioxidants and artificial flavorings as per recommended standards for ready to use therapeutic foods. The present formulation is also free from table sugar, instead it contains dried fruits for sweet taste, making it a future candidate for the management of diabetic conditions among vulnerable individuals in low income countries like Tanzania. The texture of the present formulation is a fine paste that can be squeezed easily into the mouth by a child or by his/her caregiver as per international standards (USDA, 2012). Furthermore, peanut was not included (despite its availability) because of safety concerns associated with reported aflatoxin contamination in groundnuts (Duclercq, 2014).

The LP analysis showed that it is possible to design a novel RUF formulae at a relatively low cost using locally available ingredients. The ingredient price of the proposed formulation is USD 0.15/100 g, which is lower than that of Plumpy'nut by approximately 50%. The lower price of the present formulation is due to relatively low cost of the selected ingredients. All ingredients in the present formulation can be sourced locally in Tanzania throughout the year with minimal transport and logistic costs. The opposite is true for the typical ready to use therapeutic foods, such as Plumpy'nut and Plumpy'doz. The cost of ingredients in these commonly used ready to use foods is high. Most commonly used ready-to-use foods are manufactured from vegetable oil, peanuts, milk powder, sugar and micronutrient supplements (Brixi, 2018). Peanuts and milk powder account for over 50% of product mass and for the total cost of ingredients (Brixi, 2018). The global supply chain, transport and logistics for most commonly used ready to use foods are costly. Ingredients for these foods are typically

shipped to the global manufacturing sites, mainly in France and the United States, and then transported to communities in need (Bazzano *et al*., 2017).

The UNICEF analysis of the supply chain of Plumpy'nut to Africa shows that air transportation is often required, bringing transportation cost to 39% of the product landed cost (UNICEF, 2009). Intellectual property protection and licensing has raised the cost and constrained the global supply of typical ready to use therapeutic foods like Plumpy'nut (Bakhsh, 2012). In fact, the high cost of commonly used ready to use therapeutic foods has been found as the primary reason for donors' inability to meet the basic nutrition needs in developing countries (UNICEF, 2009). Cost constraint of the typical ready to use foods prompted us to develop an alternative formulation, which takes advantage of the availability of ingredients and the possibility of localizing production close to communities in need. With the aid of linear programming (LP) tool, the study managed to design an alternative formulation with elevated content of preformed omega-3 DHA and EPA, amino acids, minerals and essential cofactors for the ALA conversion, all of which are precursors to neurotransmitters.

The downside of the LP analysis is that nutrient data were obtained from different sources that are likely to have some discrepancies in ingredients' nutritional values. Some data were obtained from Tanzania Food Composition Tables, which currently does not contain all nutrient data for the identified ingredients. The missing nutrient composition for the candidate ingredients were imputed from other sources such as USDA nutrition database, Canadian Nutrient File, Danish Food Composition Database and peer reviewed papers. One should however, understand that nutrient composition of foods is known to vary within and across regions and countries (Merchant & Dehghan, 2006). This might have affected the present analysis, especially if the final nutrient composition of candidate foods was not accurate. Laboratory LP analysis of the nutrient values for the candidate ingredients will prevent mistakes resulting from discrepancies in ingredient nutritional value compared with current databases. Further laboratory testing of the prototype formulation could confirm accuracy of estimated essential nutrients and anti-nutrient composition.

In order to address the aforementioned limitations of the LP analysis, a product prototype was developed. Thereafter, its nutritional values were established in the laboratory. The laboratory results were then compared with that of LP analysis. The laboratory analysis confirmed that the nutritional values of the prototype formulation were within the pre-

established cutoffs established in the LP analysis (Table 2). In addition, acceptability testing was conducted to ascertain consumer perception regarding sensory qualities of the developed product. The results from sensory testing showed that almost all consumers liked sensory attributes of the product very much, confirming that the prototype formulation adequately met the sensory preferences of consumers. Similarly, the econometric analysis of this study showed that almost all primary caregivers were willing to pay at least 500 Tsh (US$ 0.15) for the prototype formulation, confirming a probable minimum retail price that was pre-estimated in the LP tool. Econometric experiment further showed that caregivers' education persists as a strong predictor of WTP for the prototype formulation. However, experiments in a large population could further validate the quality of prototype formulation and help us modify and improve the nutritional constraints of the product. Additionally, testing for true ileal protein digestibility for calculating Digestible Indispensable Amino Acid Score (DIAAS) would also validate the quality of the present optimized product. But all in all, the findings from the LP analysis and that of laboratory analysis showed that an alternative ready to use food with an elevated content of preformed DHA and EPA, as well as high quality protein can be formulated from local ingredients (other than costly dairy ingredients) for the prevention and management of nutrition related mental health illnesses.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings from this study have showed that affordable ready-to-use food rich in bioavailable omega-3 DHA and EPA as well as other essential nutrients can be developed from locally available ingredients (other than costly dairy and fish oil ingredients) for the prevention and management of nutrition related mental health illnesses. The analysis of this study further showed that it is possible to develop a product that meets UN specifications set for ready-to-use foods for prevention of under nutrition using local ingredients that can be farmed in Tanzania and other parts of East Africa. Furthermore, the analysis from this study showed that microalgae, sesame seeds, pumpkin seeds, golden berries and baobab are primary ingredients that derived the composition of preformed omega-3 DHA and other essential nutrients with high biological value in the present optimal formulation. This study successfully developed a product rich in preformed omega-3 DHA and EPA as well as cofactors for the conversion of ALA in the body. This is the first study to develop a plant-based food with elevated levels of preformed omega-3 DHA and EPA in Africa. It is also the first study to spark the need for developing omega-3 DHA dietary guideline, which is currently missing in Africa.

The product contains about 220 mg of preformed omega-3 DHA+EPA, enough to meet the RDI for children aged 2-10 years. The product can also provide adequate amount of iron, zinc, vitamin A and high quality protein, which are generally regarded as limiting nutrients in developing countries. In addition, the formulated product is naturally packed with cofactors required for the conversion of omega-3 ALA into omega-3 DHA in the body. Moreover, the product contains a relatively well-balanced omega-6 to omega-3 fatty acids (2.5:1) ratio compared to commonly used RUFs in Africa. The nutritional composition of the product meets the UN standards for phytic acid to iron ratio, phytic acid to zinc ratio, calcium to phosphorus ratio and zinc to copper ratio, which are determinants of mineral absorption in the body. It also contains more than 95% PDCAAS, which is a measure of the protein quality. The product is free from added sugar; it contains natural sweet fruits with less carbohydrate, making it a future candidate for the prevention or management of diabetes. The product contains ingredients which international organizations are currently regarding them

as future innovative foods that will benefit human nutrition and the environment. Just as this study believes, international organizations also believe that these ingredients will replace meat as they have a relatively lower emission rate of greenhouse gases compared to meat.

In the light of these findings, this product is worthy reaching as many people as possible in developing countries where prevalence of omega-3 deficiency is pervasive. In this case, a business-based approach could be the best tactic of ensuring a sustainable supply of the product in the community. As a means of executing this business idea, this study proposes a business model that encompasses smallholder farmers (mainly women group farmers), food processing centers and the public domain (government, non-governmental organizations (NGOs) and private companies) to ensure a sustainable supply of the present product to people who need it. In this business model, the study visualize that women group farmers will do the production of organic raw materials under the supervision of agricultural experts who will provide technical skills on organic farming and farming as business. The processing centers will engage themselves in processing, packaging and labeling of the product. The public domain, which may include the government and NGOs like UNICEF, WFP, WHO, GAIN, USAID and private companies will provide initial financial incentives and technical support for quick and sustainable distribution of the product to as many people as possible in Africa and other parts of the developing world. Based on the initial data on consumer acceptance and WTP experiments of this study, this product will be highly accepted in the community.

Success of this business will have direct economic outcomes through contributing to mental health and wellbeing of consumers, value addition to the identified food ingredients, which may increase incomes and productivity at farm level. Through manufacturing and distribution processes, this business will create direct employments and increase the value of indirect jobs. Furthermore, products from this study will expand the choice space and at the same time supply bioavailable omega-3 DHA and other essential nutrients that are usually inadequate in most African diets. In so doing, the products will be addressing the diet related mental retardation, which is one of the major latent causes of economic stagnation in developing countries. The business idea of this product will create a “transpiration stream” to encourage farmers increase their acreage and productivity. It will also expand the local market for the produce of the identified food ingredients and thereby improve the price and turnover of the produce locally both of which will help secure farmer’s incomes. In fact, this

business idea will significantly contribute to global efforts of improving nutrition and health, and eventually eradicate poverty, which are one of the themes highlighted in the United Nations-Sustainable Development Goals (SDGs).

5.2 Recommendations

The limited sample size (n=60) may have made it difficult to identify other determinants with statistical significance on willingness to pay for the formulated product. There is a need for another WTP study with a relatively larger sample size to substantiate all potential determinants of consumer acceptance and willingness to pay for the formulated ready-to-use food in a population. Clinical and field efficacy trials in a large population are needed to validate the quality and performance of the formulated product in ensuring nutrition and mental health of the target population. Testing for true ileal protein digestibility for calculating digestible indispensable amino acid score (DIAAS) as commonly used in feed composition tables for pig is also needed to validate the quality of the formulated ready-to-use food.

Some studies have shown that utilization of omega-3 DHA by the brain may be affected by the permeability of the blood brain barrier (BBB) on its way to the brain tissues across the brain cells. In the capillaries that form the blood–brain barrier, endothelial cells are wedged extremely close to each other, forming the so-called tight junctions. The tight gap allows only small molecules, fat-soluble molecules and some gases to pass freely through the capillary wall and into brain tissue. In this aspect, nanotechnology to Nano-encapsulate omega-3 DHA is recommended to overcome the blood brain barriers that may affect proper utilization of this bioavailable essential fatty acid in the brain. The nanotechnology in this regard should involve the use of safer Nano-carriers that have been proven effective in carrying essential nutrients to brain tissues.

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