THE DEVELOPMENT AND NUTRITIONAL COMPOSITION OF A FOOD PRODUCT COMBINING SWEET POTATOES, PEANUTS, AND CHICKPEAS TO ADDRESS VITAMIN A AND MACRONUTRIENT DEFICIENCES IN SUB-SAHARAN AFRICA.

by

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(Under the Direction of Chad Paton)

ABSTRACT

Undernutrition in sub-Saharan Africa (SSA) effects over 200 million individuals and is the underlying cause of over 50% of all childhood deaths. Vitamin A Deficiency (VAD) is one common form of undernutrition present, affecting around half of the population. Sweet potato crops have been promoted to address VAD, with a single sweet potato providing enough vitamin A, as β-carotene, to meet daily needs. However, the bioavailability of β-carotene is dependent on the presence of dietary fat. So, this work focused on developing a food product that could increase β-carotene bioavailability, as well as provide additional macronutrients, by combining sweet potatoes with peanuts and chickpeas. The final food product formulation, developed through computer modeling, resulted in a nutrient rich product, containing adequate amounts of total energy, providing all essential amino acids, and meeting the daily vitamin A needs of individuals in one or two servings of this food product.

INDEX WORDS: Vitamin A Deficiency, Orange-Fleshed Sweet Potatoes, β-carotene retention, Sub-Saharan Africa Undernutrition

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CHAPTER 1

INTRODUCTION

1.1 Background

Undernutrition, defined as the inadequate intake of essential nutrients required to perform all biological functions and maintain health, is a worldwide public health concern affecting the health of many individuals (FAO, 2017). Undernutrition is a general term that can be present as macronutrient deficiencies (total energy, protein, fat) or micronutrient deficiencies (vitamins and minerals) (Black *et al*., 2008). Over 800 million individuals worldwide are undernourished, and this number is even greater when considering underlying micronutrient deficiencies (FAO, 2017). Inadequate intake of essential nutrients leads to a variety of negative health outcomes including stunted growth, delayed mental development, impaired immune response, and early death (Black *et al.*, 2008; Black *et al.*, 2013; Dewey & Begum, 2011). Although awareness of undernutrition has increased, including being encompassed into The United Nations Millennium Development Goals and Sustainable Development Goals, progress towards reducing this problem has not been equal among nations and many remain undernourished (Black *et al*., 2008; United Nations, 2015).

Sub-Saharan Africa (SSA) is one region where undernutrition remains highly prevalent and progress in reducing this problem has been slow. SSA has the highest prevalence of undernutrition among all developing nations with almost a quarter of the population affected

(FAO, 2017). Additionally, SSA is one of the only regions where undernutrition has actually risen from 175 million in 2000 to 224 million in 2017 (FAO. 2017).

Although undernutrition is seen among all regions of SSA, the prevalence is not evenly distributed. The greatest prevalence is seen in the eastern and central regions of SSA, affecting 34% and 26% of the population in these areas (FAO, 2017). Recent unstable climate conditions, both severe flooding and droughts, as well as ongoing conflict in many of these countries has resulted in the instability of food production and availability, contributing to the rising rates of undernutrition (OECD-FAO, 2016; UNICEF Eastern and Southern Africa Regional office, 2016; FAO, 2017). The western and southern regions of SSA are also affected by undernutrition, but at much lower rates, affecting 12% and 8% of the population (FAO, 2017). Lower rates of undernutrition have been attributed to increases in agriculture production, urbanization, and implementation of nutrition programs by local governments (FAO, 2017). However, although the prevalence differs, recent data shows little to no progress in reducing the prevalence of undernutrition among all regions of SSA. Thus, there continues to be a need to aid in the reduction of undernutrition in all regions of SSA.

Children and pregnant women are most vulnerable to the negative effects of undernutrition in SSA due to increased nutrient needs to support growth (Black *et al*., 2008). Two indicators of chronic undernutrition in children are the presence of stunting and wasting (FAO, IFAD, UNICEF, WFP and WHO., 2018; Black *et al.*, 2008). In SSA, 40% of all children are stunted and 10% are wasted (FAO, 2017). Both stunting and wasting can lead to impaired mental development, low productivity, increased rates of childhood infectious diseases, and early death (Black *et al.*, 2008; FAO, 2017). Pregnant women who are undernourished have an

increased risk of adverse pregnancies outcomes including maternal and fetal deaths. In addition, abnormalities in fetal growth during pregnancy is often irreversible throughout the child's life (FAO, 2017).

1.2 Problem Statement

Vitamin A Deficiency

Among all forms of undernutrition, vitamin A deficiency (VAD) is one of the most common and severe forms present in SSA (Black *et al*., 2013; Low, Mwanga, Andrade, Carey, & Ball, 2017; Sindi *et al*., 2013; World Health Organization, 2019). Regionally, 48% of the population have low vitamin A blood levels (Stevens *et al*., 2015). Pregnant women and children are most vulnerable to the negative health consequences of VAD. VAD is the leading cause of preventable blindness, with more than three million children under the age of five having impaired vision health, including complete blindness (Yunusa *et al*., 2015). VAD also causes stunted growth and mental development, as well as poor immune function (Black *et al.*, 2008). In children, this results in increased susceptibility of childhood infectious diseases, which leads to thousands of deaths each year (Stevens *et al*., 2015).

The major causes of VAD are low intake of vitamin A rich foods and low absorption and utilization in the body (Low *et al.*, 2017). Animal-based foods are the preferred source of vitamin A because these foods contain high amounts of preformed vitamin A retinyl esters, which are completely absorbed and utilized in the body (Harrison, 2012). However, due to low availability and access of these foods in in SSA, less than 10% of individuals caloric intake is consumed from animal products (van Berkum, Achterbosch, Linderhof, Godeschalk & Vroege, 2017). Rather, most of individuals diet is comprised of plant-based sources, predominantly cereal grains

and starchy crops including maize, rice, wheat, and cassava (van Berkum *et al*., 2017). These crops are rich in energy but contain little to no vitamin A. Additionally, the vitamin A form that is found in plant-based products, predominantly existing as β-carotene, is less bioavailable than the form in animal-based food products and is dependent on a multitude of biological and environmental factors (Bechoff & Dhuique-Mayer, 2017). So, when addressing VAD in these regions, considering both the availability of vitamin A rich foods and bioavailability is important.

The increased promotion and production of orange-fleshed sweet potatoes (OFSP) in SSA has been one method used to address VAD in these regions. OFSP have been promoted because of these crops' rich β-carotene content, with a single sweet potato being able to meet the daily requirements of most individuals (Hotz *et al.*, 2012). Additionally, the consumption of these crops has already shown positive results in increasing vitamin A blood levels in children (van Jaarsveld *et al*., 2005). The International Potato Center (CIP) has been a key part in the distribution of these crops, as well as educating farmers and caregivers on proper agriculture practices and post-harvest cooking methods (International potato center, 2017; Hotz *et al.*, 2012; Low *et al*., 2017). OFSP crops have been grown primarily for at-home consumption, however, recent utilization of OFSP in secondary food products has been effective in increasing the overall economic contribution of these crops, reducing post-harvesting losses, and increasing the availability of vitamin A rich foods year around (Hagenimana *et al.*, 2001; Kapinga, Tumwegamire, & Ndunguru, 2007; Low *et al.*, 2017; Low & van Jaarsveld, 2008). Thus, the continued promotion of these types of secondary food products can aid in the long-term sustainability of the use of OFSP to reduce VAD.

Macronutrient Deficiencies

Macronutrient deficiencies are other prominent forms of undernutrition in SSA (Black *et al*., 2008; FAO, 2017; FAO, IFAD, UNICEF, WFP and WHO, 2018). Inadequate intake of all macronutrients (carbohydrates, fat, protein) results in one non-specific form of macronutrient undernutrition referred to as total energy deficiency.

Macronutrients primary function is to provide energy, expressed in food as kilocalories (kcal) or kilojoules (kJ), to support all cellular activities. Thus, when the intake of all macronutrients is low, energy will not be available for cells to perform properly. In SSA, the major cause of low energy intake is the result of overall low access and availability of food (Food and Agriculture Organization of the United Nations, 2000). Energy deficits range from 100 to 400 calories per day, which is among the highest of all regions in the world (Food and Agriculture Organization of the United Nations, 2000). In children, chronic deficits in these ranges contribute to high rates of stunted growth, impaired mental development, and increased susceptibility to infectious diseases (Black *et al*., 2008; FAO, 2017).

A second form of macronutrient undernutrition is protein deficiency (FAO, 2007; Schönfeldt and Hall, 2012). Protein deficiencies can coexist with an energy deficiency or standalone. Although protein can provide energy for cells, it performs many additional functions including muscle growth, tissue maintenance, enzymatic reactions, transportation of biological molecules, and immune function (Institute of Medicine, 2005). In order to perform these functions, protein is continuously being synthesized in the body from individual amino acids (Institute of Medicine, 2005). There are twenty amino acids that make up the proteins in the body, nine of which must be consumed through dietary sources. Inadequate intake of total

protein or any one of these essential amino acids inhibits protein synthesis. This can lead to health consequences that effect almost all biological functions including stunting, muscle wasting, and poor immune function (Institute of Medicine, 2005).

Protein deficiencies are common in SSA as a result of low availability of protein rich food sources (FAOSTAT, 2007; Schönfeldt and Hall, 2012). Animal-based products contain all the essential amino acids and in adequate amounts, but due to limited access to these foods, protein is predominantly consumed from plant-based sources (Schonfeldt & Hall, 2012; Young & Pellet, 1994). Many of these plant-based foods are low in total protein, as well as are limited in some of the essential amino acids, specifically lysine (cereals) and sulfur-containing amino acids (tubers) (Oke, Redhead, & Hussain, 1990; Schonfeld & Hall, 2012). Additionally, access to complimentary food products containing these essential amino acids (i.e. animal-based products or legumes) is also limited in these regions, which also contributes to insufficient intake of these amino acids (Stephenson *et al*., 2010). As a result, deficiencies of total protein and these specific essential amino acids are common in many areas of SSA.

1.3 Project Description

This study was aimed at addressing VAD through the development of a food product that combined sweet potatoes with additional regionally promoted crops. The combination of these crops, each varying in nutrient composition, also allowed for the development of a single product that addressed multiple forms of macronutrient undernutrition. Most importantly, the nutrient synergy between these crops can allow for increased β-carotene bioavailability and thus effectiveness in addressing VAD.

The specific objectives of this research included:

1. Using a nutrient database, develop an ingredient formulation combining sweet potatoes with additional regional food sources, which provide differing macronutrient contents (carbohydrates, protein, fat), to meet the recommended daily allowance (RDA) of vitamin A retinol activity equivalent of β-carotene. In addition, by combining sweet potatoes with additional regional food sources with differing macronutrient contents, develop a formulation that also provides all the essential amino acids in ratios that are in line with reference amino acid scoring patterns, and 300 calories (15% of daily calorie needs based on a 2,000-kcal diet) per serving.
2. Produce a food product and validate that the RDA of vitamin A equivalent β-carotene is provided in this product.
3. Assess whether a significant loss of β-carotene will occur from thermal sterilization.

Hypothesis:

The theoretical vitamin A content of a food product produced from a formulation of sweet potatoes, peanuts, and chickpeas will be within 10% of the actual vitamin A of the food product before and after thermal sterilization processing, providing the recommended daily allowance (RDA) of vitamin A equivalent of β-carotene.

CHAPTER 2

LITERATURE REVIEW

2.1 Vitamin A

Chemical Structure

Vitamin A is a fat-soluble vitamin that consists of a group of retinoid compounds (figure 1) including all-trans-retinol, all-trans-retinal, retinoic acid (RA), and retinyl ester (retinyl palmitate) (O'byrne, 2013). All-trans-retinol is most commonly referred to as vitamin A and is also the form that is transported in the blood (O'byrne, 2013). Retinal and its isomers, are found in the eye tissues and are responsible for maintaining adequate vision health (O'byrne, 2013). RA exists as all-trans-retinoic acid and 9-cis retinoic acid, each functioning in genetic regulation (O'byrne, 2013). Lastly, all-trans-retinyl ester is the storage form of vitamin A in the body, primarily stored in the liver (O'byrne, 2013). Together, these retinoids allow for the many biological functions performed by vitamin A.

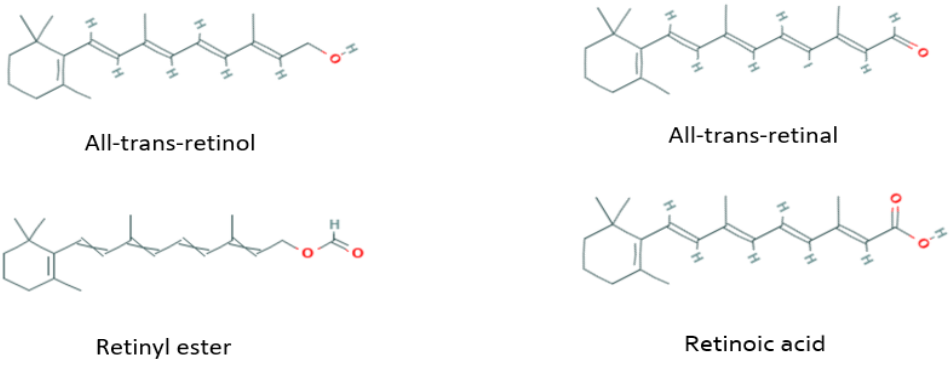

Figure 1. Retinoid Forms of Vitamin A. Figures obtained from PubChem and The National Library of Medicine (NLM). All figures are within public domain. Figures were accessed at: National Center for Biotechnology Information. PubChem Database. Retinol, CID=445354, https://pubchem.ncbi.nlm.nih.gov/compound/445354 (accessed on Apr. 3, 2019)

Dietary Sources of Vitamin A

The human body is unable to synthesize vitamin A, thus it must be obtained from dietary sources. The first form of vitamin A that is found in the diet exists as preformed vitamin A retinyl esters (retinyl palmitate) (Linus Paulding Institute, 2015). Preformed vitamin A retinyl esters are most commonly found in animal-based products such as liver, egg yolks, and dairy products. In addition, many ready to eat cereals have been fortified with preformed vitamin A retinyl esters (Harrison, 2012). In the body, retinyl esters are hydrolyzed into the active form of vitamin A, all-trans-retinol, absorbed in the enterocytes, and then transported in the lymph to be further processed in the liver (Harrison, 2012). Retinyl esters from animal sources are completely metabolized and either immediately used or stored for later use in the body. For this reason, excess intake of preformed vitamin A retinyl esters can lead to toxic levels in the body (Linus Paulding Institute, 2015).

In addition to retinyl esters found in animal-based products, provitamin A carotenoids, chemical compounds found in plant-based food sources, can be hydrolyzed in the body to provide the body with vitamin A (Linus Paulding Institute, 2018). There are three major provitamin A carotenoid compounds found in plants- β-carotene, α-carotene, and β-cryptoxanthin (Linus Paulding Institute, 2018). Of these, β-carotene (Figure 2) is the most prominent form found in nature, as well as has the greatest bioavailability in the body (Linus Paulding Institute, 2018). Rich sources of β-carotene include carrots, sweet potatoes, pumpkin, kale, and spinach (Linus Paulding Institute, 2018). Compared to preformed vitamin A, the absorption and utilization of β-carotene in the body is less efficient and dependent on many factors in the body, including the presence of vitamin A and the need in the body (Harrison,

2012). For this reason, there has shown no risk of toxicity with excess intake of β-carotene, even when intake levels exceed four times the recommended daily intake of vitamin A (Dimitrov *et al.*, 1988). The majority of vitamin A consumed in SSA is in the form of β-carotene, so for that reason, the focus of this paper will be on β-carotene utilization vitamin to provide A rather than preformed animal sources (Low *et al.*, 2017).

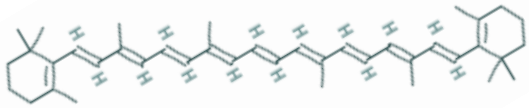

Figure 2. Chemical Structure of β-carotene. Obtained from the PubChem Database/National Library of Medicine (NLM) which is within public domain. Information was accessed at: National Center for Biotechnology Information. PubChem Database. beta-Carotene, CID=5280489, https://pubchem.ncbi.nlm.nih.gov/compound/5280489 (accessed on Apr. 3, 2019).

Metabolism of β-carotene

The conversion from β-carotene to the biologically active form of vitamin A is a multi-step process (Harrison, 2012). In order for β-carotene to be absorbed by the enterocyte, it must be released from the food matrix and then formed into a micelle (Harrison, 2012). β-carotene is stored in matrixes of plants, and the intracellular location of these matrixes (i.e. chloroplasts in leaves, chromoplasts in fruits), can impact the disruption and release of β-carotene in the intestinal tract of humans (van het hof, West, Weststrate, & Hautvast, 2000). Following the release of β-carotene, free β-carotene is formed into a micelle (i.e. a fat molecule) (Harrison, 2012). Micelle formation is essential for the solubilization of β-carotene and allows for the absorption by enterocyte cells (Reboul, 2013). The formation of these micelles requires the presence of bile salts and pancreatic juices, as well as additional lipid compounds including cholesterol, phospholipids, and triglycerides (Reboul, 2013). Together, these lipid compounds with β-carotene form a single fat molecule.

Once inside of a micelle, β-carotene is absorbed in the enterocytes through multiple protein transporters, located primarily in the upper half of the small intestine (Harrison, 2012). A few of these transporters that have been found to aid in the absorption include scavenger receptor class B type I (SR-B1), apolipoprotein B (apoB), cluster determinant 36 (CD36), and lipoprotein lipase (LPL) (Harrison, 2012).

Following absorption into the enterocyte, β-carotene can be 1) symmetrically cleaved by beta-carotene-15,15'-monooxygenase 1(BCO1), 2) asymmetrically cleaved via beta, beta-carotene 9',10'-oxygenase (BCO2), or 3) remain intact (Harrison, 2012; Shete and Quadro, 2013). The path that β-carotene takes is dependent on various factors including the rate of BCO1 (Harrison, 2012). BCO1 activity has been found to be affected by vitamin A blood levels, as well as the presence of additional dietary components (Harrison, 2012). If β-carotene is cleaved via BCO1, all-trans-retinal, also known as retinaldehyde, results. All-trans-retinal can be further oxidized by retinal dehydrogenase to form retinoic acid (RA) (Harrison, 2012). The newly formed RA primarily acts as a gene regulator by acting as a ligand for both retinoic acid receptor and retinoid X receptor (Harrison, 2012). All-trans-retinal can also be reduced by retinal reductase (RALF) to produce all-trans-retinol, a biologically active form of vitamin A (Harrison, 2012). The end products of asymmetrical cleavage by BCO2 are believed to be utilized primarily as antioxidant molecules within the mitochondria and do not contribute to vitamin A synthesis.

All-trans-retinol is the form of vitamin A that is further transported throughout the body. This transportation occurs by first forming retinyl esters through the enzyme lecithin: retinol acyltransferase (LRAT) (Reboul, 2013). These newly formed retinyl esters are circulated through the body with support from lipoprotein molecules called chylomicrons (Reboul, 2013). Retinyl

esters are incorporated in the hydrophobic core of these complexes, along with additional lipid molecules and intact β-carotene (Shete & Quadro, 2013). These chylomicron complexes enable vitamin A retinoids to be transported throughout the lymph to the liver where it is either stored or further processed to be sent to external tissues (Harrison, 2012).

Bioavailability and Retention of β-carotene

The bioavailability of β-carotene is greatly affected by individual genetic factors. BCO1 has been found to have many genetic variations which are associated with high and low converters of β-carotene into retinol (Harrison, 2012). The presence of vitamin A in the body can also down-regulate the transcription of BCO1, reducing the activity when RA is high and increasing activity when RA is low (Harrison, 2012). As a result, low levels of vitamin A in the blood have resulted in an increased absorption of β-carotene in the body and high levels of vitamin A in the blood have prevented absorption (Harrison, 2012).

Another factor that affects the bioavailability of ingested β-carotene is the presence of dietary fat. This has been shown in-vitro, where β-carotene combined with as little as 1% fat resulted in increased micelle formation (Huo, Ferruzzi, Schwartz, & Failla, 2007). In gerbils, both an increased micelle formation and conversion of β-carotene into retinol was seen when 6% and 12% fat was added to orange-fleshed sweet potato (OFSP) powder (Mills *et al*., 2008). The bioavailability of β-carotene has also been shown to increase in humans, with a 75% increase in bioavailability with the addition of oil to mashed OFSP (Van Jaarsveld *et al*., 2005). The effect of added fat is not restricted to OFSP, as increased bioavailability in humans was also found when fat was added to kale, spinach, lettuce, and carrots (Brown *et al*., 2004; Jalal, Nesheim, Agus, Sanjr, & Habicht, 1998; Jayarajan, Reddy, & Mohanram, 1980; Tawanda, Kyung-Jin,

Bermudez, Guangwen, & Hlanganiso, 2017; Van het hof, Tijburg, Pietrzik, & Weststrate, 1999). Although the exact amount of fat needed to increase absorption is still unknown, as little as 2.4 grams of fat in a β-carotene-rich meal has been shown to increase the bioavailability of β-carotene in individuals (Ribaya-Mercado *et al*., 2007). Thus, even minimal amounts of fat can greatly enhance the absorption and utilization of β-carotene in the body.

The type of fat has also been shown to affect the bioavailability of β-carotene. The addition of peanut butter, rich in monounsaturated fat, versus lard, rich in saturated fat, was studied in kale (Tawanda *et al*., 2017). β-carotene was converted to retinol at greater rates when kale was consumed with peanut butter compared to consuming kale with lard (Tawanda *et al.*, 2017). Micelle formation also increased greater with the addition of monounsaturated rich oils compared to oils higher in polyunsaturated fats (O'Connell *et al*., 2008). The greater bioavailability with monounsaturated fats has been explained by the ability of monounsaturated fat to have a greater ability to form micelles and chylomicron in the body, as well as follow a similar absorption process to β-carotene (Tawanda *et al.*, 2017; van Greevenbroek, van Meer, Erkelens, & de Bruin, 1996).

The food matrix is an additional dietary factor that impacts the bioavailability of β-carotene in the body. β-carotene is found inside a matrix of plants, which allows for proper storage and resistance to chemical changes when exposed to heat, light, and oxidation (Bechoff & Dhuique-Mayer, 2017). However, these matrices can limit the release of these carotenoids in the body, decreasing absorption and utilization (Bechoff & Dhuique-Mayer, 2017). The matrices also differ among plant sources, which have also been shown to affect the bioavailability. When comparing various β-carotene-rich foods, leafy greens consistently show much lower conversion

rates to retinol in the body compared to broccoli, peas, carrots, and other mixed β-carotene meals (Castenmiler *et al.*, 1999; van het hof *et al.*, 1999; van het hof *et al.*, 2000).

Food processing can disrupt these matrices, causing an increase in the bioavailability of β-carotene (Bechoff & Dhuique-Mayer, 2017). Physical destruction (i.e. cutting, chopping), has been shown to destroy these matrices, allowing for the release and utilization in the body (Bechoff & Dhuique-Mayer, 2017). Thermal food processing can also induce these changes. Rock *et al.*, 1998 found that heat processed carrots and spinach showed greater increases of blood vitamin A levels compared to the raw form of these vegetables (Rock *et al.*, 1998). Similarly, OFSP that were boiled and deep fried also showed significantly higher bioavailability compared to raw OFSP (Tumuhimbise, Namutebi, & Muyonga, 2009).

However, food processing can also negatively impact β-carotene content in food through degradation. Degradation of β-carotene, primarily through cis/trans isomerization and oxidation, occurs when exposed to high heat treatment, light, and oxygen (Penicaud *et al.*, 2011). This is attributed to the all-trans double bond chemical structure of β-carotene that is most abundantly found in plant-based food (Figure 2). In high-temperature food processing, isomerization is common, resulting in high amounts of cis-isomers in secondary food products (Marx, Stuparic, Schieber, & Carle, 2003; Penicaud *et al.*, 2011; von Doering, Sotiriou-Leventis, & Roth, 1995). This degradation not only reduces the overall content of β-carotene, but cis-isomers have also been found to have a lower conversion rate to vitamin A compared to all-trans-β-carotene (Thurnham, 2007). Thus, food processing that minimizes these losses should be considered.

Some common processing methods of β-carotene-rich crops include drying, boiling, and baking (Low *et al.*, 2017). β-carotene retention in processing was studied in OFSP and found that

among these processing methods, boiling, baking, and steaming resulted in the greatest retention of β-carotene, retaining around 70-90% of the original amount (Bechoff & Dhuique-Mayer, 2017; De Moura, Miloff, & Boy, 2015). On the other hand, drying of sweet potatoes, especially under high temperature and long periods, retained only 60% of the β-carotene (De Moura *et al.*, 2015). Furthermore, the extended storage of these dried products also resulted in the further reduction to as low as 20% of the original β-carotene amount (De Moura *et al.*, 2015). Biofortified maize and cassava crops also showed significant decreases of β-carotene following drying, and in some cases showed a complete loss of β-carotene (De Moura *et al.*, 2015).

Additional thermal food processing, including sterilization and pasteurization, is also commonly used in the processing of β-carotene-rich crops into secondary food products. The effect of these methods on β-carotene degradation in spinach and carrot juices and purees showed minimal degradation of β-carotene, retaining greater than 75% of the original content (Rock *et al.*, 1998). This was attributed to shorter processing times needed for both types of thermal processes (Marx *et al.*, 2003; Rock *et al.*, 1998). However, as temperature increased to above 130^{o} Celsius, greater degradation was seen (Marx *et al.*, 2003). There is limited research on various types of food products following this specific processing, thus β-carotene retention must be considered when undergoing these processes.

Functions of Vitamin A

Vitamin A plays a crucial role in vision health. Retinol aids in the formation of rhodopsin, a pigment located inside the rods of the retina, which aids in low light vision (Linus Paulding Institute, 2018). The formation of rhodopsin occurs through the isomerization of all-trans-retinol into 11-cis-retinal, which then binds to a protein known as opsin, forming

rhodopsin. In the presence of light, 11-cis-retinal isomerizes into all-trans-retinal, detaches from opsin and gives off electronic energy to form the pictures seen by the eye. Rhodopsin is continuously being regenerated, thus adequate amounts of retinol are needed to enable this process to continue.

Vitamin A also performs many functions involved in gene expression and cellular differentiation (i.e. determining the function of unspecialized cells) (Linus Paulding Institute, 2018). The form of vitamin A that predominantly performs this function is RA (Linus Paulding Institute, 2018). RA binds onto retinoic acid receptors (RAR) and retinoic X receptors (RXR), which together forms a heterodimeric complex. This complex binds onto retinoic acid response elements (RAREs) on the promoter region of mRNA and regulates gene transcription. Over 500 genes have been shown to be regulated through this complex, thus plays a key role in the development and maintenance of many tissues in the body (Linus Paulding Institute, 2018).

Vitamin A has specifically been associated with the regulation of many genes that impact embryonic development. The role of RA and its receptors on genetic regulation of embryo development has dated back to the 1930's, showing maternal vitamin A deficiency associated with abnormalities in fetal growth and early death (Mason, 1935). More recent work shows that RA is involved in the central nervous system development of embryos through assisting in brain patterning and segmentation, as well as neuron development of the spinal cord (Clagett-Dame & Knutson, 2011; Maden, 2007; Maden, Gale, Kostetskii, & Zile, 1996). RA also aids in heart development through compartmentalization and valve formation, as well as in skeletal, kidney, and uterus development (Clagett-Dame & Knutson, 2011; Dersch & Zile,1993; White, Highland,

& Clagett-Dame, 2000). Thus, the need for vitamin A through all stages of embryo development is necessary for the appropriate development of all organs.

Lastly, vitamin A plays a role in immune function (Linus Paulding Institute, 2018). One way that vitamin A impacts immunity is through the genetic regulation and cellular differentiation of epithelial tissues (i.e. skin, eyes, lung, nose, and gastrointestinal tract). Vitamin A is required for the differentiation of goblet cells, which are mucus-secreting cells on epithelial tissues (Semba, 1998). These cells are necessary to keep outside tissues moist and aid in providing the first line of defense for the body.

In addition to maintenance of epithelial cells, vitamin A and RA are involved in antigen recognition immunity primarily through T-cell differentiation (Beijer, Kraal, & den Haan, 2014; Duriancik, Lackey, & Hoag, 2010; Ross, 2012). Unspecified T-cells are activated through the presence of antigens and cytokines (signaling proteins), which differentiate T-cells into specific T helper (Th) cells (i.e Th1, Th2, Th17, and Treg). Each of these specified cells responds to different environments through the production of specific cytokines. Th1 and Th17 respond to intracellular pathogens, Th2 responds to extracellular pathogens, and regulatory T-cells (Treg) regulates these responses to protect from excess inflammation (Ross, 2012). Vitamin A has been found to aid in the increased transcription and differentiation of Th2 and Treg cells and decreases the differentiation of Th1 and Th17 response. So, RA is essential for appropriate immune response and prevention against excess inflammation (Ross, 2012).

Vitamin A requirements

Vitamin A requirements are expressed in Retinol Activity Equivalents (RAE) to account for the variability of bioavailability of vitamin A (National Institute of Health, 2010). RAE

values are based on conversion factors of the various forms of vitamin A compared to retinol. The conversion factors between β-carotene and RAE has been determined through testing the bioavailability of β-carotene in pure oil and then comparing those results to the bioavailability of β-carotene in foods (Sauberlich *et al*., 1974). β-carotene in pure oil had an average conversion factor of 2 μg β-carotene to 1 μg of retinol (Sauberlich *et al*., 1974). The bioavailability of β-carotene in various carotenoid-containing foods showed larger variability, but on average, 6 μg β-carotene to 1 μg of retinol was determined as an appropriate conversion factor (Haskell *et al*., 2004; Ribaya-Mercado *et al*., 2007; Tang *et al*., 1999; van het hof *et al*., 1999). Taking the product of the bioavailability of β-carotene in oil (1 to 2 μg β-carotene) and that of food (1 to 6 μg β-carotene), a conversion factor of 1 μg retinol to 12 μg β-carotene was determined. Table 1 shows the current conversion factors of β-carotene, along with the additional types of provitamin A carotenoids.

The target daily intake of vitamin A is based on both age and gender of an individual. These values are reported in Table 2 as Recommended Dietary Allowance's (RDA). RDA are reference nutrient values that are estimated to meet 97-98% of all healthy individuals.

Tolerable upper limits (UL's) have also been set for vitamin A due to its ability to be stored in the body. However, vitamin A consumed in the form of β-carotene poses no risk for adverse outcomes with excess intake due to the body's ability to inhibit absorption. Only minimal yellowing of the skin with no further signs of toxicity has been shown in humans following daily intake of 45 mg β-carotene /day, exceeding four times the recommended amount (Dimitrov *et al*., 1988).

Vitamin A Deficiency (VAD)

VAD negatively affects the health of an individual in multiple ways including impaired vision health. Without adequate vitamin A in the body, rhodopsin is unable to be regenerated in the eye, leading to impaired low light vision and night blindness (Linus Paulding Institute, 2018). In addition, decreased maintenance of epithelial tissues around the mucous membrane of the eye, leads to the increased secretions of keratin, a fibrous protein, which results in white spots referred to as bitot spots (Linus Paulding Institute, 2018). Long-standing deficiency can lead to the further buildup of keratin and hardening of the cornea called xeropthalmia (Linus Paulding Institute, 2018). Xeropthalmia can lead to night blindness as well as complete blindness (Cohen *et al.*, 1985; Sommer, West, & Ross, 1996).

VAD is also associated with impaired embryo development. Through the use of vitamin A depleted and retinoid ligand knockout animal models, VAD has seen to cause abnormalities in brain development and patterns, decreased neuron development of the spinal cord, insufficient valve formation in the heart, respiratory system defects, and underdeveloped kidneys (Dersch & Zile,1993; Maden, 2007; Maden *et al*., 1996; Mark, Ghyselinck, & Chambon, 2009; Smith, Dickman, Power, & Lancman, 1998). Birth defects in humans have also been shown with VAD, including cleft palate. Low vitamin A levels during pregnancy (<800 μg RAE) was associated with 50% increased risk of an infant being born with cleft palate (Johansen, Lie, Wilcox, Andersen, & Drevon, 2008). The results of these studies show that RA is essential for embryo development, and insufficient intake of vitamin A leads to major negative health outcomes.

Another consequence of VAD is a weakened immune function. Decreased maintenance of epithelial tissues causes increased secretions of keratin, resulting in hardened skin surfaces

referred to as keratinization (Linus Paulding Institute, 2018; Tseng, Hatchell, Tierney, Huang, & Sun, 1984). In addition, VAD can lead to decreased mucus secretions of epithelial cells, which allows bacteria to enter the body that would normally be trapped in the mucus-secreting cells (Semba,1998; Stephenson, 2001; Tseng *et al*., 1984). VAD weakens the adaptive immune response by decreasing the differentiation and activation of Th2-cells and increases the activation of Th1 cells. As a result, antibody response is decreased, and inflammation is increased, severely impacting proper immune response (Stephenson, 2001).

2.2 Vitamin A Deficiency in Sub-Saharan-Africa

Prevalence of VAD in SSA

Among all developing countries, SSA has one of the greatest prevalence's of VAD (Stevens *et al*., 2015; World Health Organization, 2019). In 2013, 48% of the population had low vitamin A blood levels (Stevens *et al*., 2015). Furthermore, the prevalence is above 60% in some countries including Central African Republic, Dem. Republic of Congo, Somalia, and Liberia (Stevens *et al*., 2015). VAD is highest among women of childbearing age and children under the age of five, with 10% and 40% of these individuals have low vitamin A blood levels (<0.70 micro mol/L) (Black *et al*., 2013).

VAD has led to many major health consequences in SSA. Over three million children under the age of five have impaired vision health, including complete blindness as a result of VAD (Yunusa *et al*., 2015). VAD also decreases immune function, which has contributed to increased risk and severity of infectious diseases, including measles, malaria, and diarrheal diseases in SSA (Stevens *et al*., 2015). As a result of these diseases, VAD is an underlying cause

to thousands of child deaths each year (Stevens *et al.*, 2015). Furthermore, childhood morbidity can be reduced by 25% just through VAD prevention methods (United Nations Children's Fund, 2007). Thus, addressing VAD in SSA is essential to improve the health of many individuals, and reduce childhood morbidity.

2.3 Methods to Address VAD in SSA

Vitamin A supplementation has been one method used to address VAD in SSA. Vitamin A supplementation has been made possible through government support and supplement donations from United Nations Children's Fund (UNICEF), World Health Organization (WHO), and Canadian government organizations (United Nations Children's Fund, 2007). Vitamin A supplementation is performed through the distribution of two high dose vitamin A capsules annually to replenish vitamin A liver stores (United Nations Children's Fund, 2007). When received in adequate amounts (two annual supplements), childhood morbidity has been shown to be reduced by up to 25% (United Nations Children's Fund, 2007). However, less than half of the highly affected countries in SSA receive effective coverage levels to show these results, with the lowest coverage among rural and lower socioeconomic communities (United Nations Children's Fund, 2007). Additionally, the cost of a supplementation program requires continued external support and is hard to sustain (MOST USAID Micronutrient Program, 2004). For this reason, supplementation is a quick response to a complex health issue and is not a practical, long-term, sustainable method.

A more sustainable method to address VAD has been through agriculture-based methods. One agriculture-based approach that has been utilized to address VAD is through biofortification

of crops (Bouis & Saltzman, 2017). Biofortification involves the breeding of crops to enhance specific genetic qualities that improve nutrient content or agriculture growth. Varieties of maize, cassava, and sweet potato have all been fortified with β-carotene, with sweet potatoes being one of the most common and promising crops to utilize in these regions (Bouis & Saltzman, 2017).

Biofortification of sweet potatoes has resulted in varieties of OFSP that are both nutritionally and agriculturally adequate for SSA. Over forty different varieties of OFSP have been developed through research organizations including HarvestPlus, International Potato Center (CIP), and National Agriculture Research and Extension System (NARES) ("Extract from 2014 catalogue of OFSP for Africa", 2014). Many of these varieties contain as much as 700 µg RAE of β-carotene per 100 grams (Hotz *et al.*, 2012). This is more than double the amount in pumpkin and greater than eight times the content of some dark leafy green and mango plants (Hotz *et al.*, 2012). In addition, these OFSP varieties are agriculturally favorable for farmers in SSA, being able to withstand varying environmental conditions, easy to grow, and can be easily distributed through vegetative propagation (i.e. vines can be cut from current plants and distributed to farmers) (Low *et al.*, 2017). As a result, OFSP has been thought as a promising crop to increase the availability of vitamin A rich crops.

The use of agriculture-based projects has been able to increase the availability, production, utilization, and consumption of OFSP. Projects conducted primarily under the CIP have worked to distribute thousands of OFSP vines to farmers and educate them on proper growing techniques (Hagenimana *et al.*, 1999b; International potato center, 2017; Low, 1997; Low *et al.*, 2007; Low *et al.*, 2017; Sindi, Kiria, Low, Sopo, & Abidin, 2013). Following this distribution and education, over 90% have continued to grow OFSP in the following years

(International potato center, 2017; Low *et al*., 2007). Caregivers have also been educated on proper cooking and storage techniques to increase crop utilization (Low, 1997). This has resulted in increased consumption of OFSP by 60% and overall daily vitamin A intake by up to 85% (Hotz *et al*., 2012). Furthermore, the consumption of boiled and mashed OFSP by children showed significant increases in vitamin A biomarkers, specifically liver stores (van Jaarsveld *et al*., 2005). Similar studies confirm that the use of agriculture-based initiatives of OFSP significantly improved vitamin A blood levels (Bovell-Benjamin, 2007; Low *et al*., 2007; Low *et al*., 2017). The acceptance of the crops by farmers and increased vitamin A blood levels after consumption show that this OFSP utilization is a promising method to decrease the prevalence of VAD.

There are still many challenges in the effectiveness of agriculture-based methods to address undernutrition. As with supplementation, accessibility of crops to all farmers remains a major limitation of these initiatives (Low *et al*., 2017). The distribution of vines has been predominantly through farmer groups in urban communities, missing many rural and lower socioeconomic farmers (Laurie, Faber, & Claasen, 2018; Mudege, Mayanja, & Muzhingi, 2017). An additional challenge is limited resources to adequately store and process these crops. Raw OFSP do not last very long, thus proper processing and storage are required in the form of drying, freezing, secondary food processing, etc. However, with limited infrastructure, water, and electricity in the communities, many of these processes are unable to be performed (Laurie *et al*., 2018). Limited storage capacity and seasonal dependence also lead to periods of high and low vitamin A availability, also contributing to VAD (International potato center, 2017). Lastly, without a market need, the economic contribution of these crops is limited, resulting in lower

adoption by farmers to continue growing these crops (Laurie *et al*., 2018). Thus, addressing post-harvest processing and utilization is an essential component to result in a sustainable approach to address VAD.

A way to address these challenges has been through the use of food-based approaches, focusing on secondary food product production with OFSP. To date, a few products have been developed utilizing OFSP crops. OFSP have been processed into a flour and then incorporated into baked goods, including chips, doughnuts, and breads (Laurie & Van Heerden, 2012; Laurie *et al*., 2018; Low & van Jaarsveld, 2008). OFSP have also been incorporated into infant weaning products, nutritional supplementary products, and porridge dishes (Amagloh & Coad, 2014; Gebretsadikan, Bultosa, Forsido, & Astatkie, 2015; Kunyanga, Imungi, Okoth, Vadivel, & Biesalski, 2012). A supplementary product was produced from the combination of OFSP flour with various legumes, cereals, vegetables, and fish powder to provide a variety of nutrients and 35% of vitamin A requirements (Kunyanga *et al*., 2012). In addition, Gebretsadikan *et al*., 2015 produced a porridge dish with OFSP flour, legumes, and vegetable leaves, which also resulted in a protein and energy-rich product. Additionally, this product was highly accepted by individuals when at least 65% OFSP was used in the product (Gebredsadikan *et al*., 2015). Furthermore, CIP has developed a shelf-stable OFSP puree that has been shown to retain higher levels of β-carotene compared to OFSP flour (International potato center, 2017). All of these products have allowed for the further use of these crops to increase availability, shelf stability, and economic growth (Mudege *et al*., 2017).

However, the major limitation of current food-based products utilizing OFSP is the overall retention of β-carotene. OFSP flour is most commonly used in many of these food

products (Gebretsadikan *et al*., 2015; Kunyanga *et al*., 2018). Drying of OFSP to produce this flour has shown to significantly decrease β-carotene content, compared to other processing methods (Bechoff & Dhuique-Mayer, 2016; De Moura *et al.*, 2015). This can significantly impact the amount of total vitamin A in these final products. Thus, the type of processing methods utilized in the production of these products must also be considered to effectively use food-based approaches in addressing VAD.

2.4 Importance of Macronutrients

Background and Functions of Macronutrients

Macronutrients are essential nutrients that are the primary source of energy for the body. Macronutrients include carbohydrates, fats, and proteins. Upon consumption of these nutrients, the body breaks down each into smaller molecules, primarily glucose from carbohydrates, single fatty acid chains from fats, and amino acids from proteins, which all can be oxidized into chemical energy that can be used by the cells (Institute of Medicine, 2005). The energy that is provided from these macronutrients is measured in kilojoules (kJ) or kilocalories (kcal).

Carbohydrates are needed in the greatest amount of all the macronutrients and are the body's main source for immediate energy (Institute of Medicine, 2005). For each gram of carbohydrate consumed in the diet, 17 kJ or 4 kcal of usable energy is produced from oxidative processes (Institute of Medicine, 2005). Carbohydrates can be stored in the body in limited amounts. The storage form of carbohydrates is glycogen and is primarily stored in the muscles (~500 grams) and the liver (~100 grams). These stores are quickly depleted after a 24-hour fast,

thus a continuous intake of carbohydrates is essential to ensure a constant energy supply for biological functions (Jensen, Rustad, Kolnes, & Lai, 2011).

Carbohydrates are present in the diet as either simple or complex carbohydrates (Institute of Medicine, 2005). Simple carbohydrates consist of monosaccharides (glucose, fructose, and galactose) and disaccharides (sucrose, lactose, maltose). These sugars are found in a wide variety of foods including fruit, vegetables, milk, and many baked goods. These sugars are digested and converted into glucose quickly in the body, providing a rapid source of energy for the body (Institute of Medicine, 2005). On the other hand, complex carbohydrates, made up of long chains of monosaccharides molecules, are metabolized much slower and provide a longer supply of energy (Institute of Medicine, 2005). Complex carbohydrates are found in many products including breads, pastas, potatoes, rice, and cereals. Both types of carbohydrates are important to maintain adequate health and support all biological functions.

Fats, or lipids, are a second group of essential macronutrients that provide energy for the body. Fats can supply a larger amount of energy in the body, providing 37 kJ and 9 kcal per gram (Institute of Medicine, 2005). Unlike carbohydrates, fat can be stored in unlimited amounts in the body, which allows for a constant energy supply during fasting periods (Institute of Medicine, 2005). Fat also performs functions involved in fat-soluble nutrient transportation, cell membrane structure, neural development and function, and cardiovascular health (Institute of Medicine, 2005).

Fat in the diet is primarily found as triglycerides, which are compounds made up of an alcohol glycerol backbone and three hydrocarbon side chains, referred to as fatty acids (Institute of Medicine, 2005). Two major types of fat are found in foods, saturated and unsaturated fats.

These two types of fat differ by the makeup of these hydrocarbon side chains. In saturated fats, every carbon is single bonded to a hydrogen atom in each of the fatty acid chains. Dietary sources of saturated fats are found in animal-based products such as meat, poultry, milk, butter, and cheeses. On the other hand, unsaturated fats contain hydrocarbon chains that include one or more double bonds. Unsaturated fats that are made up of fatty acids containing one double bond are referred to as monounsaturated fats. Monounsaturated fats are rich in canola and peanut oils, avocados, nuts, and seeds. Polyunsaturated fats are unsaturated fats that contain fatty acids with two or more double bonds. Similarly, polyunsaturated fats are found in many types of oils, nuts, seeds, and fish in the diet.

The third macronutrient in the diet is protein. Protein is the most abundant macronutrient in the body besides water (Institute of Medicine, 2005). Protein is the building blocks of the body and is what makes up many tissues, transport molecules, enzymes, hormones, and antibodies (Institute of Medicine, 2005). Together, all these proteins are involved in an array of functions including body movement, growth, chemical reactions, and immune response (Institute of Medicine, 2005). In addition, dietary protein can be oxidized for energy if carbohydrate or fat intake is low, providing 17 kJ or 4 kcal per gram (Institute of Medicine, 2005).

All protein, both in the body and in food, is composed of long polypeptide chains of amino acids (Institute of Medicine, 2005). There are twenty different amino acid compounds that are used to make up proteins. Eleven of these amino acids can be synthesized in the human body and are considered nonessential. The other nine amino acids cannot be made in the body thus must be obtained from dietary sources. These nine amino acids, referred to as essential or indispensable amino acids, are histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

threonine, tryptophan, and valine (Institute of Medicine, 2005). For the body to perform all the necessary functions, all essential amino acids must be present in adequate amounts.

The body can synthesize proteins from these amino acids in a process referred to as protein synthesis. Protein synthesis is made up of two processes, transcription and translation (Institute of Medicine, 2005). Transcription begins in the nucleus of each cell where deoxyribonucleic acid (DNA), which contains the genetic code for every protein in the body, is copied into messenger ribonucleic acid (mRNA) and then carried out of the cell into the cytoplasm. This is where the second step, translation, begins. Translation begins by mRNA attaching onto a molecule called a ribosome, which is where protein synthesis ultimately occurs. The ribosome reads the mRNA in sets of three nucleotides, referred to as a codon. Each codon is specific to an amino acid. When a codon is read, a second type of RNA, called transfer RNA (tRNA), carries that specific amino acid to the protein building site on the ribosome. The ribosome attaches the amino acid onto a second amino acid forming a peptide bond. This process continues to repeat forming a long polypeptide chain of amino acids. Once the protein is complete, a specific codon on the mRNA communicates to the ribosome that the protein is complete and allows for the protein to be detached from the ribosome. The new protein completes any further processing and then is ready to be transported out through the body (Institute of Medicine, 2005).

Many foods consumed in the diet contain some protein but differ in the amount and composition of essential amino acids (Institute of Medicine, 2005). Complete protein food sources contain all the essential amino acids and in sufficient amounts where protein synthesis can occur without any missing amino acids. For this reason, intake of complete protein food

sources is the preferred source of dietary protein to ensure the intake of all essential amino acids (Institute of Medicine, 2005). Sources of complete proteins are primarily found in animal-based foods, including meat, eggs, and milk products. Plant-based foods, including many cereals, vegetables, and legumes, also contain protein but are referred to as incomplete protein sources (Institute of Medicine, 2005). Incomplete protein sources contain some of the essential amino acids in adequate amounts but are low in other essential amino acids, which are referred to as limiting amino acids. To meet protein needs from these sources, a variety of plant-based foods must be consumed and in adequate amounts.

The limiting amino acids in plant-based foods is determined by the lowest available essential amino acid in the ratio needed for protein synthesis (Institute of Medicine, 2005). Thus, if an essential amino acid is not present in the body, it will completely stop, or limit protein synthesis from continuing past that point. As shown in Figure 3, since no lysine is present, it is the limiting amino acid, and the excess valine and cysteine amino acids shown are unable to be utilized for protein synthesis. In foods, the most common limiting amino acids include lysine in cereal grains and sulfur amino acids (cysteine and methionine) in legumes and tuber crops. Table 3 shows the comparison between these most limiting amino acids between food groups.

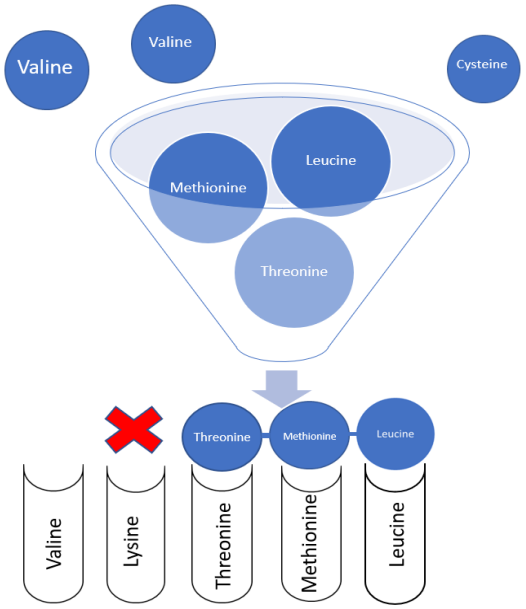

Figure 3. Effects of Limiting Amino Acids on Protein Synthesis. This figure shows how a limiting amino acid works to prevent protein synthesis. The funnel depicts the body utilizing amino acids present to build protein chains. However, no lysine is present, thus the chain cannot continue, and the protein cannot be built. The valine and cysteine are excess amino acids in the body, unable to be utilized in protein synthesis.

Macronutrient Requirements

Macronutrient requirements are expressed in multiple ways (Institute of Medicine, 2005). Individual RDA values for each macronutrient has been determined for individual age and gender. Secondly, Acceptable Macronutrient Distribution Ranges (AMDR) expresses requirements by giving the percentage of each macronutrient that should be consumed each day relative to each macronutrient. A third way macronutrient recommendations can be expressed are as estimated energy requirements (EER). EER is determined through equations that calculate energy needs by determining an individual's basal metabolic rate, thermal effect of food, and physical activity (Institute of Medicine, 2005). Energy ranges obtained from these equations are listed in Table 4. These recommendations are based on reference heights and weights for each age and gender group.

To meet daily protein needs, RDA reference values are often used. The RDA for total protein intake has been primarily determined through nitrogen balance studies (Institute of Medicine, 2005). These studies look at the amount of protein, or nitrogen, that must be consumed to equal the amount lost in the body through feces, urine, and sweat. To account for additional protein needs for growth in children and during pregnancy, recommendations have been determined through nitrogen balance studies with additional requirements accounting for protein deposited into tissues (Institute of Medicine, 2005). Table 5 shows RDA for total protein intake for specific age and gender groups.

In addition to total daily protein needs, daily essential amino acid requirements have also been set to ensure that all amino acids are present in adequate amounts. Total daily requirements for each essential amino acid has been determined through nitrogen balance studies and stable isotopic tracer methods. Requirements are based off of individuals' age and weight (Table 6). Since the protein found in food is not all equal in the type and amount of amino acids present, and any limiting amino acid will halt protein synthesis, an essential amino acid scoring pattern has also been developed. A reference amino acid scoring pattern was developed by taking the requirement of each essential amino acid and dividing it by total protein needs (Institute of Medicine, 2005). Different food sources can easily be compared to this reference scoring pattern to determine if all essential amino acids are present in adequate amounts. The reference amino acid pattern, expressed in mg/g of protein, is shown in Table 7.

2.5 Macronutrient Deficiencies in Sub-Saharan Africa

Prevalence of Macronutrient Deficiencies

Insufficient energy, or calorie intake, is a common non-specific macronutrient deficiency in SSA (FAO, 2017; Food and Agriculture Organization of the United Nations, 2000). Over 30%, or close to 250 million individuals in SSA do not meet daily energy needs with calorie deficits ranging from 100 to 400 calories per day (Food and Agriculture Organization of the United Nations, 2000). On average, calorie consumption for SSA is around 2,100 kcal per day (FAO, 2007). However, these values greatly range among the region of SSA. For example, western and southern Africa have much higher average calorie intake, ~2,500 and 2,850 kcal/day (FAO, 2007). On the other hand, central and eastern Africa have average calorie intakes only at around 1,760 and 1,950 kcal/day (FAO, 2007). Additionally, calorie intake for SSA is much lower compared to other countries in the world including western Asia, >3,000 kcal/day, and other less developed counties, ~2,800 kcal/day (FAO, 2018).

Protein deficiency is another widespread form of macronutrient undernutrition in SSA (FAO, 2007; FAO, 2017; Schönfeldt and Hall, 2012). Protein deficiency can either coexist with an energy deficiency or be present alone (FAO, 1997). On average, individual protein intake is only around 44 grams/caput/day in many areas of SSA (FAO, 2018). One major reason for the low protein intake is the large proportion of food intake coming from cereal grains and tubers, specifically maize and cassava (FAOSTAT, 2007). These crops are composed almost entirely of starch, but limited in essential nutrients, including total protein and all the essential amino acids (Sayre *et al*., 2011). Cereal crops are limited in lysine and tubers are limited in sulfur-containing amino acids (i.e. cysteine and methionine) (Oke, Redhead, & Hussain, 1990; Schonfeld & Hall,

2012; Young & Pellet, 1994). As a result, continued intake of these foods limited in these essential amino acids will result in the inability to synthesize proteins, and major health consequences can result.

Consequences of Macronutrient Deficiencies in SSA

Both energy and protein deficiencies have severely impacted the health of many individuals, especially children in SSA. Stunting, low height for age, affects over 40%, or 50 million children under five years old (FAO, 2017). Stunting is most common in a child's first few years of life and results from inadequate energy or protein during pregnancy or throughout infancy and early childhood (Muller & Krawinkel, 2005). Wasting, or low weight for height, is another consequence of macronutrient deficiency, affecting over 12 million children in SSA (Muller & Krawinkel, 2005). Wasting usually occurs with rapid and acute energy and protein deficiency, causing the body to break down body fat and muscle to supply energy needs for metabolic processes (Muller & Krawinkel, 2005). Consequences of stunting and wasting in children have led to impaired brain development, low energy, decreased immune function, and death (Dewey and Begum, 2011; Unicef, 2013).

Strategies to Address Macronutrient Undernutrition in SSA

Community-based health programs have been one of the strategies used to address macronutrient deficiencies and undernutrition in SSA. These programs address the underlying health-related causes of undernutrition in the most vulnerable populations, women and children (United Nations Children's Fund, 2015). The promotion of breastfeeding, adequate complementary feeding practices, health education, sanitation, and adequate health care are just some of the many programs that have been implemented (United Nations Children's Fund,

2015). The continued promotion and support for these health programs is essential to aid in the prevention of undernutrition and improve the overall health of individuals.

Agriculture-based approaches have also been key to addressing macronutrient undernutrition in SSA. Agriculture production is the primary contributor to economic growth, especially among smallholder farmers (OECD/FAS, 2016). Thus, to increase food and energy intake, increasing agriculture production is one method that can be used. However, not only must food be available, but specifically nutritious food. As a result, the increase of specific nutrient-rich crops has been targeted to increase agriculture production and diversity.

Legumes are one type of crop that has been used to increase diet diversity in SSA (United Nations Children's Fund, 2015). Legumes are a family of plants including beans, peas, lentils, and groundnuts. Legumes are rich in energy, protein, and micronutrients, especially iron and zinc (USDA, 2019). Legumes rank as one of the highest protein containing plant-based crops, containing around 20% protein, compared to less than 10% for many cereals and tubers (Young & Pellett, 1994). In addition, legumes many of the essential amino acids that are limiting in cereal crops, including lysine (Snapp, Rahmanian, & Batello, 2018). This makes legumes a great complimentary protein food source with high cereal food-based diets.

Oilseed production, specifically groundnut production, is a specific sub-group of legumes that has significantly increased in production over the past decade (OECD/FAS, 2016). Groundnut production ranks as the fifth most produced crop in SSA with the majority being produced in the west and east regions of SSA (Snapp *et al.*, 2018). Groundnut production has been popular among smallholder farmers because of its hardiness through unstable environmental conditions, showing the lowest yield reduction with limited rainfall. Groundnuts

are also popular due to these crops rich energy content (primarily from fat), and versatility in cooking and consumption methods (Daryanto, Wang, & Jacinthe, 2015; HarvestChoice, 2018, OECD/FAS, 2016, Snapp *et al.*, 2018). Recent, genetic advances in groundnut varieties have been able to increase agriculture output, nutrient content (i.e. oleic acid), and shelf stability (Janila *et al*., 2016). As a result, these improved variations have even shown increased income in areas of Uganda (Menale, Bekele, & Geoffrey, 2010).

A second sub-group of legumes that has increased in production and is predicted to continue to increase among all regions of SSA are pulses (OECD/FAS, 2016; Snapp et al, 2018). Pulses are a legume that produces a dry grain for consumption (OECD/FAS, 2016; Snapp *et al*., 2018). Pigeon pea is a pulse that has been widely adopted in southern and eastern regions of Africa, with significant increases in production in Malawi and Mozambique (Snapp *et al*., 2018). In addition, disease-resistant varieties of both chickpeas (Ethiopia) and cowpeas (Western Africa), are other types of pulses that have been promoted in SSA (Snapp *et al.*, 2018). All pulses are also popular among smallholder farmers as a result of their hardiness in drought-like conditions, low cost, and ability to be intercropped with maize (OECD/FAS, 2016; United Nations Children's Fund, 2015). For this reason, pulses can be a valuable crop to increase diet diversity, and specifically protein.

Table 1:

Retinol Activity Equivalent (RAE) Conversion Factors of Vitamin A[a]

1 RAE =	1 µg retinol
1 RAE =	12 µg β-carotene
1 RAE =	24 alpha-carotene and beta-cryptoxanthin

a. Values obtained from: National Institute of Health Office of Dietary Supplements, 2010.

Table 2:

Recommended Dietary Allowance for Vitamin A in µg RAE[a]

Age Range (years old)	Males	Female
0-1	400-500[b]	400-500[b]
1-3	300	300
4-8	400	400
9-13	600	600
14-18	900	700
19-30	900	700
31-70	900	700
>70	900	700
Pregnancy	N/A	750
Lactating	N/A	1200

a. Values Obtained from: Food and Nutrition Board, Institute of Medicine. Vitamin A, 2001.
b. Infant recommendations are based off of Adequate Intakes because of inadequate data to make RDA recommendations. Adequate Intakes have been made off of the mean intake of vitamin A consumed from breastmilk

Table 3:

Comparison of Common Limiting Amino Acids in Foods[a,b]

Food Group	Lysine	Sulfur amino acids[c]	Threonine	Limiting amino acid
Animal foods	85	38	44	N/A
Cereals	31	37	32	Lysine
Legumes	64	25	38	Sulfur containing amino acids
Vegetables	49	24	35	Sulfur containing amino acids

a. Source: United States Department of Agriculture, Agriculture Research Services, 2019.
b. Values expressed in mg/g of protein.
c. Sulfur containing amino acids include methionine + cysteine

Table 4:
Daily Estimated Energy Requirements in Kilocalories (kJ)[a,d]

Age Range (years old)	Female[b,c]	Male[b]
1-3	1000-1400 (3200-3900)	1000-1400 (3500-4200)
4-7	1200-1800 (4100-4900)	1200-1800 (4400-5200)
8-12	1400-2200 (5200-6400)	1400-2400 (5500-7000)
13-18	1600-2400 (6700-7300)	2000-3200 (7500-9400)
19-50	1800-2400 (6100-7500)	2400-3000 (7700-9500)
>50	1600-2000 (6000-6900)	2000-2800 (7000-8100)

a. Energy intakes are based on Estimated Energy Requirements (EER) equations using average height and weights for each age range. For children, these are average heights and weights for each age range. Adult reference weights are 5 feet 4 inches and 126 pounds for women and 5 foot 10 inches and 154 pounds for males.
b. Calorie ranges reflect differences of physical activity levels. Calorie ranges may differ slightly depending on individual physical activity.
c. Energy requirements are for nonpregnant and lactating women. For pregnant women calorie needs for the first trimester are similar to that of nonpregnant women. An average of an additional 350 and 450 calories are need for the second and third trimester
d. Values obtained from: Institute of Medicine-Dietary Reference Intakes, 2005.

Table 5:
Recommended Daily Allowance of Protein[a]

Age Range (years)	Protein (g/day)
Infants and Children	
0-1	9-11 (AI)
1-3	13
Males	
4-8	19
9-13	34
14-18	52
>18	56
Females	
4-8	19
9-13	34
14-18	46
>18	46

a. Values obtained from: Institute of Medicine of the National Academies, 2005.

Table 6:
Recommended Essential Amino Acid Requirements Expressed as mg/kg/day[a]

	Age Group (Years old)				
	1-3	4-8	9-13	14-18	>18
Histidine	15	12	12	11	10
Isoleucine	27	23	22	21	20
Leucine	54	44	44	42	39
Lysine	45	35	35	33	30
Methionine + Cysteine	22	17	17	16	15
Phenylalanine+ Tyrosine	40	30	30	28	25
Threonine	23	18	18	17	14
Tryptophan	6.4	4.8	4.8	4.5	4.0
Valine	36	29	29	28	26

a. RDA values obtained from: WHO/FAO/UNU Expert Consultation, 2007.

Table 7:
Recommended Essential Amino Acid Scoring Patterns for Individuals >1 Years Old[a]

Amino Acid	Mg/g protein
Histidine	15
Isoleucine	30
Leucine	59
Lysine	45
Methionine and cysteine	22
Phenylalanine and tyrosine	38
Threonine	23
Tryptophan	6
Valine	39

a. Values obtained from: WHO/FAO/UNU Expert Consultation, 2007.

CHAPTER 3

METHODS

3.1 Initial Product Design

Initial ingredient formulations were developed using The Food Processor Nutrition and Fitness Software (2016) by esha Research (version 11.3.285) located at the University of Georgia's Food and Nutrition Department. The ingredient titles used from this software were Sweet Potatoes, baked in skin, w/ salt, mashed (Supplier-USDA, ESHA code-5994), Peanut butter, creamy (Supplier-USDA, ESHA code-4627), and Chickpeas, canned, drained (Supplier-USDA, ESHA code-38880). The nutrient content of each ingredient is shown in Table 8.

Table 8:
Nutrient Content of the Raw Ingredients per 100 Grams[a]

	Sweet Potato, mashed	Peanut Butter	Chickpeas
Calories (kcal)	92	598	139
Total Fat (g)	0.2	51	2.8
Total Protein (g)	2	22	7
Vitamin A (μg RAE)[b]	961	0	0
β-carotene (μg)	11,531	0	14
Iron (mg)	0.69	1.74	1.07
Zinc (mg)	0.32	2.51	0.63
Histidine (mg)	40	520	200
Isoleucine (mg)	70	580	300
Leucine (mg)	120	1,450	510
Lysine (mg)	80	640	480
Methionine (mg)	40	250	90
Cysteine (mg)	30	220	100
Phenylalanine (mg)	110	1,130	380
Threonine (mg)	110	490	260
Tryptophan (mg)	40	220	70
Valine (mg)	110	740	300

a. Data obtained from the The Food Processor Nutrition and Fitness Software (2016) by esha Research (version 11.3.285). Date accessed: October 2017.
b. RAE=Retinol Activity Equivalents. 12 μg β-carotene is equivalent to 1 RAE

Product formulations were developed and tested by varying the percentage of sweet potato, peanut butter, and chickpea, in a 250-gram serving. 250 grams was used as the reference serving size and is equivalent to a single serving of each of the individual components using the USDA exchange system (i.e. sweet potato puree=100 grams (1/2 cup), peanut butter= 60 grams (2 tablespoons), chickpeas= 90 grams (1/2 cup)) (The American Diabetes Association and the American Dietetic Association, 1995).

Determining the final product formulation was done by analyzing the nutrient content of each of the tested formulations. The primary nutrients that were analyzed were calories, total vitamin A, and all the essential amino acids. Table 9 contains the nutrient content of these target nutrients for all formulations tested. These nutrients were then compared to target reference levels, determined using RDA reference values.

- The target level for vitamin A was 900 μg RAE, which provides 100% of the RDA for most healthy individuals.
- Total calories were used to measure the overall energy and macronutrient content of each formulation, with a target value of 300 kilocalories per serving. This would provide 15% of an individual's daily calorie needs based off an average daily requirement of 2,000 kilocalories.
- Each essential amino acid was analyzed separately, with the goal of including all the essential amino acids in the final product formulation. Additionally, since the proportion of each essential amino acid in a food is also crucial, an amino acid scoring pattern, which compares the essential amino acid composition of a food source to a reference essential amino acid pattern was also used (Institute of Medicine, 2005). The reference

essential amino acid pattern is expressed in mg/g of protein, and each formulation was compared to this reference amino acid pattern, shown in Table 10.

- Total protein, fat, iron, and zinc were also observed.

From this nutrient analysis, the ingredient formulation of 60% sweet potato, 5% peanut butter, and 35% whole chickpeas met all the target levels for total calories, vitamin A, and essential amino acids, and was used to produce the final food product.

3.2 Product Development

Raw Ingredient Processing

Mississippi grown raw whole sweet potatoes (Edmuondson Farms, MS., Pack Date: 10/2/2018, Purchased Date: 10/7/2018), Crazy Richard's 100% Natural creamy peanut butter, and Goya canned whole ChickPeas were all purchased from a local grocery store in Athens, Georgia. Whole sweet potatoes were washed with skins on for 30 seconds. Potatoes were sliced into 1-inch slices using a Hobart food slicer. Potato slices were then placed in a steam cabinet and heated at 95°C for 10 minutes. Keeping the skins on, the heated potato slices were processed in a Cuisinart food processor (Model No. FP-12DCN) for 30 seconds until completely pureed. The peanut butter was stirred in the jar until the oil was uniformly distributed throughout the container. Goya canned whole ChickPeas were placed in a strainer and washed under cold water for 30 seconds. The skins were removed and then the chickpeas were patted dry.

Food Product Processing

The final food product was produced from the sweet potato puree, peanut butter, and chickpeas using the product formulation of 60% sweet potato, 5% peanut butter, and 35%

chickpeas. Each sample was made in a 250-gram serving size, which included 150 grams sweet potato puree, 12.5 grams of peanut butter, and 87.5 grams of whole chickpeas. The ingredients were all weighed using a digital scale (Denver Instrument, Model No. XP-300) and were all placed into a single bowl. All three ingredients were stirred continuously for 1 minute to combine.

Packaging

One 250-gram sample of the product was placed in multiple 50 ml Sterile Centrifuge Tubes. The product was packed tightly in the tubes, leaving 1 inch from the top of the tube. 2 ml of glycerol was then pipetted on the top of the sample. Lids were placed tightly on the tubes and were immediately placed in a -80^{o} C freezer. All other samples were placed into flexible tri-laminate three sealed 4.75”x 7.25” flat retort pouches. Each pouch was filled with a single 250-gram sample. The food sample was at room temperature (20-25^{o}C) when placed into the pouches. The pouches were then sealed using a Packworld USA heat sealer (Model No. A17-2993). Additional pouches were prepared with attached thermocouples. A hole was made in the center of one side of the pouch and a thermocouple was placed through the hole. A whole chickpea was placed on the tip of the thermocouple in the inside of the center of the pouch. This was done by piercing the center of the chickpea with the tip of the thermocouple. Gorilla glue was placed at the bottom of the chickpea to keep it on the thermocouple throughout the processing. 250 grams of the food sample was then placed in these pouches and heat sealed, similarly to the other pouches.

Heat Sterilization Processing

Heat sterilization was performed in a FMC corporation Steritort Rotary Sterilizer (Serial no. 3608 46 71) located at the University of Georgia's Food Science and Technology department. The pouches were placed in a metal cage that contained 32- 0.75-inch-wide compartments. Each pouch was placed standing up in separate compartments. The metal cage was placed on a steel beam located at the center of the retort to prevent rotation during processing. Two additional pouches with thermocouples connected to them were also placed in separate compartments in the metal cage. The thermocouples were connected to a connector site on the retort, which was then connected to an external data logger.

The sterilization process followed the overkill sterilization method, based on a 12 $D_{121^{o}C}$ reduction, or a 12-log reduction of microorganisms (Ramaswamy and Marcotte, 2006). This method calculates thermal sterilization using a $D_{121^{o}C}$ value, the time needed to reduce 90% of microorganisms at 121^{o}C, and a z-value, the temperature difference that results in a ten-fold variation of bacterial destruction, of target organisms (Ramaswamy and Marcotte, 2006). The target organism was *Clostridium botulinum* because of the final food product having a low pH (~6). Previous meta-analysis of *Clostridium botulinum* has shown appropriate $D_{121^{o}C}$ and z-values to be 0.2 and 10^{o}C. Thus, these values were used to determine lethality of *Clostridium botulinum* in this product (Diao, André, & Membré, 2014).

The sterilization process of the pouches began with a preheating step, which involved injecting steam into the retort until an internal temperature of 90^{o}C was reached. After this step, the retort was opened and the metal cage with the samples was placed in the center of the retort and the door was closed. The retort was filled with hot water and steam until both the retort and

pouches reached 121°C. Once the pouches reached 121°C (250°F) and 15 psi, the pouches were thermally heated for 4 minutes. After heating, the pouches were cooled down by applying a cold-water spray to the pouches before opening the retort. Pouches were immediately placed in a -80°C freezer until further nutrient analysis. The use of proper freezing for storage, as well as packaging in tri-laminate plastic pouches to prevent light exposure, allowed for optimal conditions for the retention of β-carotene.

3.3 Nutrient Analysis

Macronutrient, Iron, and Zinc Analysis

Samples were sent out to Merieux NutriSciences (Gainesville, FL.) in triplicate for chemical nutrient analysis of macronutrients (total calories, total fat, total protein) and specific amino acids including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. Iron and zinc were also chemically tested. Total calories were calculated from total ash (AOAC 925.51A), carbohydrates (calculation), fat (AOAC 933.05), moisture (CRA MOIST.04), and protein (AOAC 992.23). Essential amino acids were measured using the USDA MSS2 (1993) method. Iron and zinc were both measured using AOAC 984.27 methods.

β-carotene Analysis

Extraction

β-carotene analysis was performed in the laboratory of Dr. Mario Ferruzzi at The North Carolina State University-Plants for Human Health Institute located in Kannapolis, NC. The raw unprocessed sweet potatoes (with skin), the unsterilized final product, and the sterilized final product were analyzed in triplicate. All extraction processes were performed under yellow light

to limit β-carotene degradation. For each sample, 100 mg of the homogenized product was placed in 15 mL falcon tubes. 100 µL of β-apo-8'-carotenal (internal standard) and 1 mL of distilled water were added to the samples and vortexed for 5 minutes. Samples were immediately placed in a covered ice cooler for 10 minutes. After being chilled, 5 mL of chilled acetone was added to the tubes as the initial extraction solvent (Amorim-Carrilho, Cepeda, Fente, & Regal, 2014). Tubes were vortexed for 5 minutes, then placed in the covered ice cooler for 5 minutes, and then centrifuged for 10 minutes at 4000 rpm. After being centrifuged, the top acetone liquid layer was transferred into another 15 mL polypropylene falcon tube and placed under nitrogen to dry. A second 5 mL aliquot of chilled acetone was added to the original falcon tube, vortexed for 5 minutes, chilled for 10 minutes, and then centrifuged for 10 minutes. This second acetone fraction was added to the first acetone fraction and again placed under nitrogen. For the last extraction, Methyl tert-butyl ether (MTBE), an organic compound made from methanol and isobutylene, was used as the extraction solvent. MTBE has been an effective solvent in increased retention of lipids (Matyash, Liebisch, Kurzchalia, Shevchenko, & Schwudke, 2008). 2 mL of MTBE was added to the original 15 ml falcon tubes, vortexed for 5 minutes, incubated on ice for 10 minutes, and centrifuged for 10 minutes at 4000 rpm. The MTBE fraction was added to the other two acetone fraction and placed under nitrogen. After drying, 2 ml of ethyl acetate:methanol, in a 1 to 4 ratio (400 µL ethyl acetate: 1600 µL methanol), was added to the tubes and sonicated for 3 minutes using an Ultrasonic cleaner (Kendall). This liquid was syringed and filtered through 0.45 mm filter and placed in 100 µL vials.

HPLC Analysis

β-carotene was measured using HPLC (waters e2695) connected to a photodiode array (pda) detector using a C30 150x2 mml column. The column temperature was 35°C and the samples were held at 8°C. Total processing time was 17 minutes with a flow rate of 0.37 ml/min. A gradient pump mode was used with a mixture of solvent A (Methanol and Ammonium acetate (98:2 volume)) and solvent B (Ethyl Acetate). The starting gradient was 95% solvent A to 5% solvent B and increased to 15% solvent B and 85% solvent A for 3 minutes. For 5 minutes the gradient increased to 80% solvent B and 20% solvent B. For 1 minute the gradient increased to 100% solvent B and remained for 2 minutes. The gradient gradually decreased back to the starting 95% solvent and 5% solvent B over 2 minutes and were held for 4 minutes. Detection of β-carotene were measured on Empower software at 450 nm. Peak areas for each sample were compared to a standard curve (r^2=0.998) and final content in grams was determined. The extraction efficiency rate was calculated using the β-apo-8'-carotenal internal standard. The peak area of a pure sample of β-apo-8'-carotenal was determined through HPLC and compared to the peak area of β-apo-8'-carotenal found in each of the samples. The final β-carotene content of the samples was then adjusted to account for percent loss during extraction.

3.4 Statistical Analysis

Percent difference was calculated to compare the predicted to the chemical nutrient content of the final product. The formula that used was (Actual-Theoretical)/(Theoretical) x 100. A paired t-test was used to test for statistical significance of β-carotene content before and after sterilization; *a priori* α was set at $p \leq 0.05$.

Table 9:
Nutrient Content of Product Formulations Using the Food Processor Nutrition and Fitness Software (2016) per 250 Grams

	Product Formulations expressed in ratio of sweet potato: peanut butter: whole chickpeas										
	75: 12.5: 12.5	60: 30: 10	60: 10: 30	60: 05: 35	50: 30: 20	50: 20: 30	40: 40: 20	40: 20: 40	40: 50: 10	30: 20: 50	30: 50: 20
Total Calories (kcal)	403	621	391	334	633	518	760	530	874	542	886
Total protein (g)	13	21	14	12	23	19	28	20	32	21	33
Total fat (g)	17	39	15	9	40	28	53	29	65	29	66
Total Vitamin A (µg RAE)	1802	1442	1442	1442	1202	1202	962	962	962	722	722
β-carotene Equivalents (µg)	21620	17296	17296	17296	14413	14413	11531	11531	11531	8648	8648
Iron (mg)	2.17	2.61	2.27	2.19	2.70	2.53	2.96	2.63	3.13	2.72	3.23
Zinc (mg)	1.58	2.52	1.58	1.34	2.60	2.13	3.14	2.20	3.61	2.28	3.69
Histidine (mg)	300	500	340	290	540	460	660	500	740	530	780
Isoleucine (mg)	410	620	480	440	670	610	800	660	870	720	930
Leucine (mg)	830	1390	920	800	1490	1250	1820	1350	2060	1450	2160
Lysine (mg)	510	720	640	620	820	780	960	880	1000	980	1100
Methionine (mg)	180	270	190	170	280	240	330	350	370	270	390
Cysteine (mg)	150	230	170	150	240	210	290	230	320	250	340
Methionine + Cysteine (mg)[a]	330	500	360	320	520	450	620	480	690	520	730
Phenylalanine (mg)	690	1100	740	640	1180	990	1430	1060	1620	1130	1690
Phenylalanine + Tyrosine (mg)[b]	1070	1800	1130	960	1910	1570	2340	1670	2680	1770	2790
Threonine (mg)	440	600	480	450	640	580	730	620	790	660	830
Trypotophan (mg)	160	240	170	150	250	210	290	220	330	220	340
Valine (mg)	530	790	570	520	840	730	990	780	1100	820	1150

a. Daily recommendations of methionine include cysteine because of the body's ability to make cysteine from methionine.
b. Daily recommendations of phenylalanine include non-essential amino acid tyrosine because of the body's ability to make tyrosine from phenylalanine

Table 10:
Essential Amino Acid Patterns of Product Formulations Compared to Reference Amino Acid Scoring Pattern Requirements, Expressed as mg/g of Protein[a]

	Reference amino acid scoring pattern	75: 12.5: 12.5	60: 30: 10	60: 10: 30	60: 05: 35	50: 30: 20	50: 20: 30	40: 40: 20	40: 20: 40	40: 50: 10	30: 20: 50	30: 50: 20
Histidine	15	23	23	25	24	24	24	24	25	23	25	24
Isoleucine	30	32	29	35	37	30	32	29	33	28	34	28
Leucine	59	64	65	66	67	66	66	66	67	65	68	66
Lysine	45	39	34	46	52	36	41	35	44	32	46	34
Methionine + Cysteine[b]	22	26	23	26	27	23	24	22	24	22	24	22
Phenylalanine + Tyrosine[c]	38	83	84	82	80	84	83	84	83	85	83	85
Threonine	23	34	28	35	38	28	31	26	31	25	31	25
Tryptophan	6	12	11	12	13	11	11	10	11	11	10	10
Valine	39	41	37	41	43	37	39	36	39	35	39	35

a. Recommended amino acid pattern obtained from WHO/FAO/UNU Expert Consultation (2007)
b. RDA levels for methionine include non-essential amino acid cysteine due to the body's ability to make cysteine from methionine.
c. RDA values for phenylalanine include the non-essential amino acid tyrosine due to the body's ability to make tyrosine from phenylalanine

CHAPTER 4

RESULTS

4.1 Theoretical Nutrient Analysis of Test Formulations

Total Energy

Table 9 (above) shows the nutrient breakdown for total energy, protein, fat, vitamin A, iron, zinc, and all essential amino acids for the tested formulations. All the formulations provided at least 300 kcal in a 250-gram serving size. Total energy ranged from 334 to 886 kcal per 250 grams. The highest energy content was in the combination of 30% sweet potato, 50% peanut butter, and 20% chickpeas, providing 886 kcal. The formulation that contained the lowest energy content was the formulation of 60% sweet potato, 5% peanut butter, and 35% chickpea, providing 334 calories per 250 grams. In general, the formulations with the greatest percentages of peanut butter had the highest calorie content.

Vitamin A

The major form of vitamin A in this product was β-carotene, expressed as β-carotene equivalents. β-carotene can be converted to retinol activity equivalents (RAE) in a 12: 1 ratio. Since RDA levels are expressed in µg RAE of total vitamin A, these units were used to determine the total vitamin A in each formulation. The total vitamin A content of the tested formulations ranged from 722 µg RAE to 1802 µg RAE. The only formulations that did not meet the target level of 900 µg RAE per 250 grams were the two formulations that contained 30%

sweet potato. So, 40%, or 100 grams, was the minimum amount of sweet potato needed in the final product to meet the vitamin A target level.

Essential Amino Acids

All nine of the essential amino acids were present in all ingredient formulations. However, when comparing the amino acid content to the reference amino acid scoring patterns, the only formulations that met 100% of the recommended amino acid pattern was the 60% sweet potato, 10% peanut butter, and 30% chickpea, 60% sweet potato, 5% peanut butter, and 35% chickpea, and the 30% sweet potato, 20% peanut butter, and 50% chickpea (Table 10). Between these formulations, the lysine and sulfur amino acid scoring patterns were greatest in the 60% sweet potato, 5% peanut butter, and 35% chickpea (Table 10).

4.2 Final formulation

The formulation of 60% sweet potatoes, 5% peanut butter, and 35% chickpeas, met all the target nutrient levels per 250-gram serving, and was used to make the final food product. The nutrient breakdown of this formula is shown in Table 11 and Table 12.

Table 11:
Nutrient Content of Selected Essential Nutrients from a Nutrient Database in the Final Formulation per 250 Grams[a]

Total Energy (kcal)	334
Total Protein (g)	12
Total Fat (g)	9
Total Vitamin A (µg RAE)	1442
Beta-carotene (µg)	17296
Iron (mg)	2.19
Zinc (mg)	1.34
Histidine (mg)	290
Isoleucine (mg)	440
Leucine (mg)	800
Lysine (mg)	620
Methionine (mg)	170
Methionine + cysteine (mg)	320
Phenylalanine (mg)	640
Phenylalanine + Tyrosine (mg)	960
Threonine (mg)	450
Tryptophan (mg)	150
Valine (mg)	520

a. Nutrient values obtained from The Food Processor Nutrition and Fitness Software (2016) by esha Research (version 11.3.285).

Table 12:
Amino Acid Scoring Pattern of Final Formulation Compared to Reference Amino Acid Scoring Pattern

	Reference amino acid scoring pattern[a]	Amino Acid Scoring Pattern of Final Formulation
Histidine	15	24
Isoleucine	30	37
Leucine	59	67
Lysine	45	52
Methionine + Cysteine[b]	22	27
Phenylalanine + Tyrosine[c]	38	80
Threonine	23	38
Tryptophan	6	13
Valine	39	43

a. Recommended amino acid pattern obtained from WHO/FAO/UNU Expert Consultation, 2007
b. RDA levels for methionine include non-essential amino acid cysteine due to the body's ability to make cysteine from methionine.
c. RDA values for phenylalanine include the non-essential amino acid tyrosine due to the body's ability to make tyrosine from phenylalanine

4.3 Product Development

Physical Characteristics

The final product resulted in a seemingly heterogenous paste product. The color was bright orange (Figure 4). The whole chickpeas distributed throughout provided a chunky texture to the smooth sweet potato and peanut butter base. The average pH was 5.6.

Figure 4. Final Food Product (Right)

Heat Sterilization Processing

The total sterilization processing time was 54 minutes, 4 minutes at 121°C, and a total lethality of 20.18 (Figure 5).

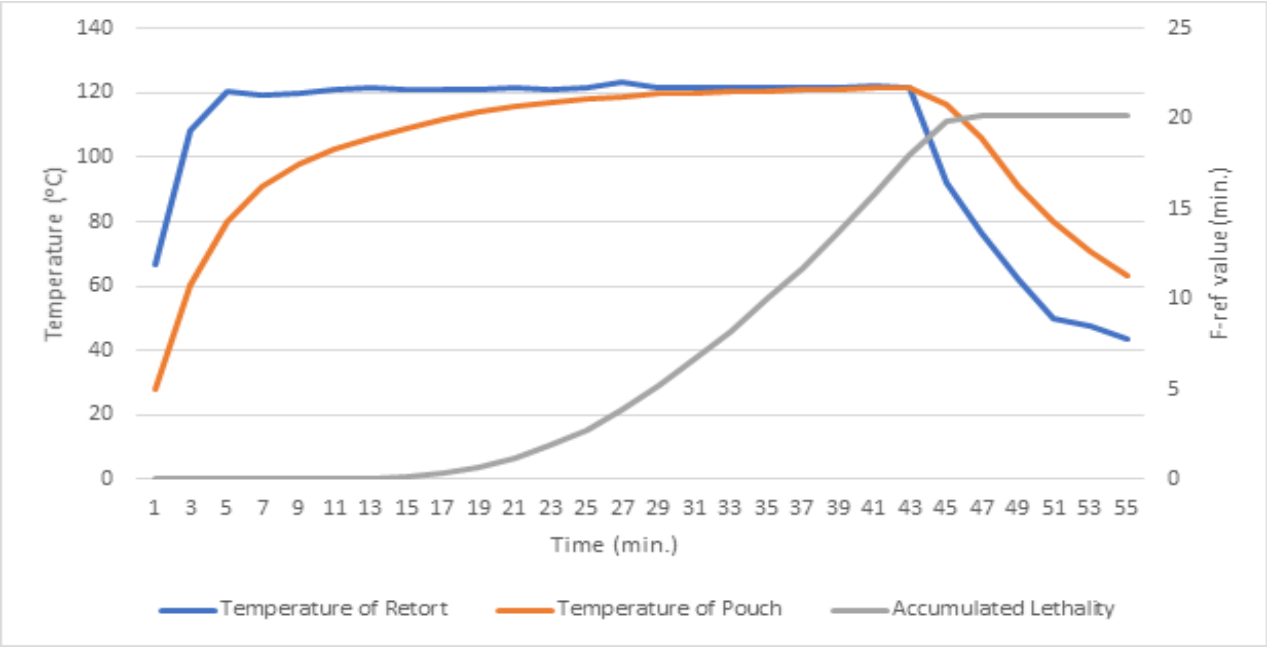

Figure. 5 Accumulated F-value and Lethality of Final Food Product Following Thermal Sterilization. Target F-value reference was 12 which was achieved around 40 minutes after processing shown by the accumulated lethality in this figure.

4.4 Chemical Nutrient Analysis

Total calories, protein, fat, iron, zinc, and specified essential amino acids were chemically analyzed by Merieux Nutrisciences. The equation used to calculate the difference among the database and actual nutrient values was: (actual-predicted)/(predicted), which has been used to compare nutrient database values (Vasilopoulou *et al*., 2003; Kafatos, Verhagen, Moschandreas, Apostolaki, & Van Westerop, 2000). All the nutrients were within 10% of the predicted value from the nutrient database except for total fat, zinc, and methionine (Table 13). However, all were within 20% of the predicted values. All the predicted values were higher than the actual content expect for histidine, isoleucine, leucine, and phenylalanine.

Table 13:
Comparison of Predicted Versus Actual Nutrient Content of Final Formulation per 250 Grams

	Predicted[a]	Actual[b]	% difference[c]
Total calories (kcal)	334	306 ± 4	-8.4%
Protein (g)	12.0	11.8 ± 0.1	-1.1%
Fat (g)	9.0	7.4 ± 0.2	-18.2%
Iron (mg)	2.2	2.0 ± 0.05	-9.5%
Zinc (mg)	1.4	1.1 ± 0.09	-16.1%
Histidine (mg)	290	292 ± 0.01	0.57%
Isoleucine (mg)	440	450 ± 0.00	2.3%
Leucine (mg)	800	825 ± 0.03	3.1%
Lysine (mg)	630	575 ± 0.03	-8.0%
Methionine (mg)	180	150 ± 0.00	-14.3%
Phenylalanine (mg)	650	675 ± 0.03	5.5%
Threonine (mg)	450	442 ± 0.01	-1.9%
Valine (mg)	520	492 ± 0.01	-5.5%

a. Values obtained from The Food Processor Nutrition and Fitness Software (2016)
b. Actual vitamin A content was from the average of triplicate samples
c. Difference between predicted and actual was calculated using the equation: (Actual-predicted)/predicted

β-carotene analysis

An 80% retention efficiency was determined (Table 14), and the values reported below for β-carotene represent adjusted values to account for the 20% loss. The β-carotene content of the raw sweet potato used to produce the final product contained 8,180 ± 66 µg β-carotene per 100 grams of fresh weight. The nutrient content obtained from the food processing nutrient software of a raw sweet potato was 8,509 µg β-carotene per 100 grams. In a single serving of the unsterilized food product (250 grams), there was 8,579 ± 213 µg β-carotene (715 ± 18 µg RAE) and 5,893 ± 135 µg of total β-carotene (491 ± 11 µg RAE) in the sterilized food product (Table 15). The unsterilized and sterilized products were statistically different, with a 31% decrease ($p=0.02$) of β-carotene in the final sterilized product. Compared to the theoretical values obtained from the nutrient database, the β-carotene content in the unsterilized food product was 50% lower and the final sterilized food product was 66% (Table 15). Additionally, the final sterilized product was the only sample that contained both trans-β-carotene (5,297 ± 124 µg) and cis-isomers (596 ± 12 µg).

True retention was measured between the unsterilized and sterilized final food product using the following equation used by van Jaarsveld, Harmse, Nestel, & Rodriguez-Amaya, 2006: (µg of β-carotene per gram of cooked food x g of food after cooking-fresh weight) / (µg of β-carotene per g of raw food x gram of food before cooking-fresh weight) x 100. True retention was 60% between the unsterilized final food product to the sterilized food product.

Table 14:
Comparison of Peak Areas of β-apo-8'-carotenal During β-carotene Extraction to Determine Extraction Efficiency

	Peak Area	Extraction Efficiency
β-apo-8'-carotenal standard	132,372	N/A
Raw sweet potato sample	105,846	80%
Unsterilized sample	105,119	79%
Sterilized sample	105,526	80%

Table 15:
Comparison of Predicted Vitamin A Content and Actual Vitamin A Content in the Unsterilized Food Product and the Sterilized Food Product[1]

	Predicted[2]	Actual[3]	% Difference[4]
	µg β-carotene (µg RAE)		
Unsterilized final product	17,296 (1,442)	8,579 +/- 213 (715)[a]	-50%
Sterilized final product	17,296 (1,442)	5,893 +/- 135 (491)[b]	-66%

1. Values with different letters indicate significant difference at P<0.05.
2. Values obtained from The Food Processor Nutrition and Fitness Software (2016).
3. Actual β-carotene content was the average of triplicate samples adjusted for percent loss.
4. Difference between the predicted and actual was determined using the equation: (Actual-Predicted)/Predicted

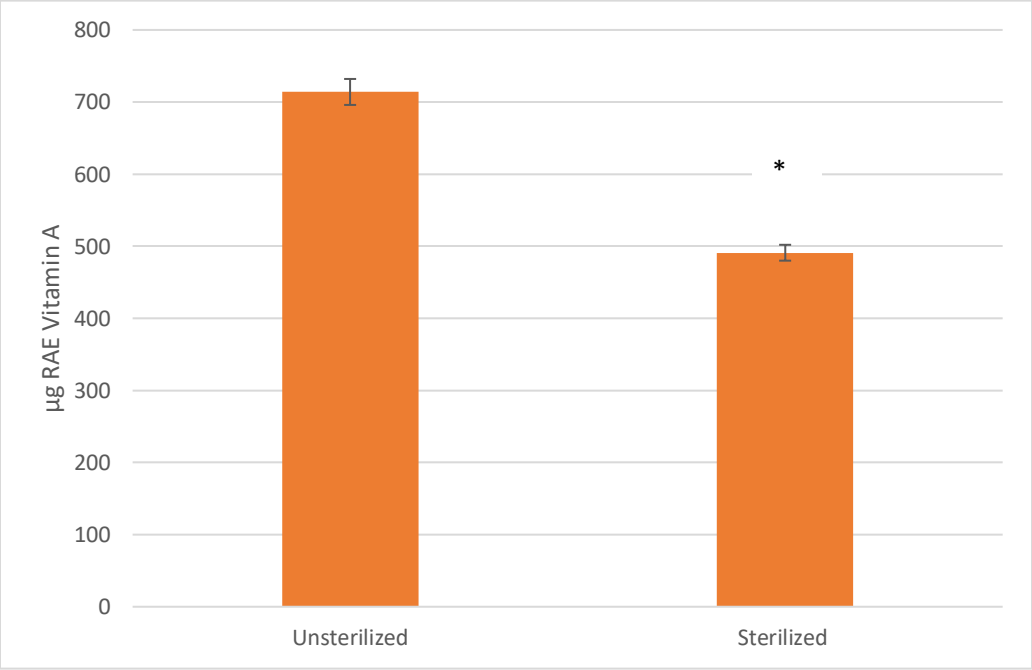

Figure 6. Total Vitamin A (µg RAE) in the Unsterilized Food Product and the Sterilized Food Product. *Indicates significant difference <0.05 between unsterilized and sterilized final product

Table 16:
Paired t-Test of β-carotene Content Before Sterilization and After Sterilization

	Unsterilized	Sterilized (with isomers)
Mean	8579	5893
Variance	136636	54917
Observations	3	3
t Statistic	7.703	
t critical value	4.303	
P value	0.02	

4.5 Comparison of Actual Nutrient Content to RDA values

The actual nutrient content obtained from the chemical analysis was compared to the RDA reference values for total energy, protein, essential amino acids, and vitamin A. One 250 gram serving of this product provides 10-33% of individuals daily energy needs (Table 17). This product provides 20% of the RDA of total protein for adults and as much as 90% for young children in 250 grams (Table 18).

Amino acid scoring patterns, expressed as mg/g of protein, were used as the reference values to determine the final formulation of this product. It was predicted that the amino acid scoring pattern of all essential amino acids met the reference amino acid scoring pattern requirements (Table 12). After chemical analysis, all the essential amino acids met these recommended patterns except methionine + cysteine (Table 19). However, the non-essential amino acid cysteine was not chemically tested in this product, and the amino acid pattern only includes the methionine content in this product.

Amino acid scoring patterns help determine protein quality of a food, but daily requirements of each essential amino acid can also be used to evaluate amino acid content. These requirements are expressed based on individuals' weight (mg/kg/day). Table 20 shows the

percentage of each amino acid provided by this food product using reference weights of various age groups. A single serving of this product provided at least a quarter of daily needs for all essential amino acids except for methionine and cysteine.

Table 17:
The Percentage of Daily Energy Requirements Provided from 250 Grams of the Final Product[a]

Age Group (years old)	Male	Female
1-3	22-31%	22-31%
4-7	17-26%	17-26%
8-12	13-22%	14-22%
13-18	10-15%	13-19%
19-50	10-13%	13-17%
>50	11-15%	15-19%

a. RDA reference values obtained from the Institute of Medicine of the National Academies, 2005.

Table 18:
The Percentage of the RDA for Total Protein Provided from 250 Grams of the Final Product[a,b]

Age Range (years old)	Percent of RDA provided from a 250-gram serving
1-3	91%
4-8	62%
9-13	35%
14-18	23-26%
19-50	21-26%
>50	21-26%

a. RDA reference values obtained from the Institute of Medicine of the National Academies, 2005.
b. Since RDA reference values are based on weight, reference weights determined by FNB/IOM were used accessed at the Institute of Medicine of the National Academies, 2005.

Table 19:
Comparison of the Actual Amino Acid Pattern to a Reference Amino Acid Scoring Pattern per 250 Grams of this Food Product

	Reference Amino Acid Scoring pattern[a] (mg/g of protein)	Amino acid pattern in food product[b] (mg/g of protein)	% of reference amino acid scoring pattern
Histidine	15	24.7	165%
Isoleucine	30	38.1	127%
Leucine	59	69.9	118%
Lysine	45	48.7	108%
Methionine + Cysteine	22	12.7[c]	58%
Phenylalanine + Tyrosine	38	57.2[d]	151%
Threonine	23	37.5	163%
Valine	39	41.7	107%

a. Reference values obtained WHO/FAO/UNU Expert Consultation, 2007.
b. Amino acid pattern determined by taking the content of each amino acid from chemical analysis divided by total protein.
c. Amino acid scoring pattern only includes the methionine present.
d. Amino acid scoring pattern only includes the phenylalanine present.

Table 20:
The Percentage of the RDA for each Essential Amino Acid (except tryptophan) Provided per 250 Grams of this Food Product[a]

	Children		Male			Female		
	1-3	4-8	9-13	14-18	>18	9-13	14-18	>18
Histidine (%)	162	122	68	44	42	66	49	51
Isoleucine (%)	139	98	57	35	30	55	40	39
Lecuine (%)	127	94	52	32	30	51	36	37
Lysine (%)	106	82	46	29	27	44	32	34
Methionine + Cysteine[b] (%)	57	44	25	15	14	24	17	18
Phenylalanine + Tyrosine[c] (%)	141	113	63	40	39	61	45	47
Threonine (%)	160	123	68	43	45	66	48	55
Valine (%)	114	85	47	29	27	46	33	33

a. RDA values for daily amino acids are expressed in mg/kg/day obtained from WHO/FAO/UNU Expert Consultation, 2007. Estimated daily needs for each age group were calculated by using reference weights determined by the FNB/IOM accessed from the Institute of Medicine of the National Academies, 2005.
b. The RDA reference values for methionine also includes cysteine due to the body's ability to make cysteine from methionine. Values in this chart only shows methionine in this product.
c. The RDA reference values for phenylalanine also includes tyrosine due to the body's ability to make tyrosine from phenylalanine. Values in this chart only shows phenylalanine in this product.

The actual vitamin A content of this final product was also compared to RDA reference values (Table 21). A single serving provided half of the daily recommended daily needs of vitamin A for all individuals except lactating women. 100% of the RDA was met for children 1-8 years old.

Table 21:
The Percentage of the RDA of Vitamin A (µg RAE) Provided per 250 Grams of this Food Product[a]

Age Group (years old)	% of RDA provided
0-1	98-123%
1-3	163%
4-8	123%
9-13	82%
14-18	55-70%
19-30	55-70%
31-70	55-70%
>70	55-70%
Pregnant	65%
Lactating	41%

a. RDA reference values obtained from the Institute of Medicine of the National Academies, 2005.

CHAPTER 5

DISCUSSION

The primary goal of this study was to develop a single vitamin A rich food product, provided in the form of β-carotene, through the utilization of sweet potatoes. Yellow and orange fleshed sweet potatoes are rich in β-carotene, and generally the darker the color, the more β-carotene it contains (Hotz *et al.*, 2012). Over 42 different varieties of OFSP have been developed for cultivation in SSA, with many of these varieties containing greater than 700 µg RAE per serving (Laurie *et al*., 2012; Hotz *et al.*, 2012; Low *et al.*, 2017). Because of these crops rich vitamin A content, as well as their ability to grow under a variety of environmental conditions, OFSP have been promoted as an effective and sustainable method to reduce the prevalence of VAD (Low *et al*., 2017). Additionally, further incorporation of sweet potatoes into secondary food product production has provided a market need, decreased crop waste, allowed for year-round availability of vitamin A, and increased the economic contribution of these crops (Hagenimana *et al*., 2001; Kapinga, Tumwegamire, & Ndunguru, 2007; Low *et al.*, 2017; Low & van Jaarsveld, 2008; Mudege, Mayanja, & Muzhingi, 2017).

Utilizing a nutrient database to develop the initial formulation was useful in this project to develop a balanced nutrient rich product. Since each of the ingredients utilized in this product contained a unique combination of the target nutrients (energy, amino acids, vitamin A), changing the amount of one ingredient in the final product, changed the composition of these three nutrients. By testing multiple variations, a final formulation that theoretically provided the

recommended daily levels of vitamin A, > 300 kilocalories, and contained all the essential amino acids was obtained. Computer modeling also assisted in achieving these recommendations in an appropriate serving size. Although nutrient databases are just estimating, this type of modeling is useful in the initial stage of developing a balanced food product.

Chemical nutrient analysis was essential in this work to validate that the estimates provided from the nutrient database were representative of what was in the actual food product. Previous studies comparing nutrient databases to actual nutrient content of food products shows that most nutrient values are within 10-20% of one another (LaComb, Taylor, & Noble, 1992; McCullough *et al*., 1999; Seljak, Stibilj, Pograjc, Mis, & Benedik, 2013). In this current project, all the nutrients tested, except for total vitamin A, were within these ranges. All nutrients except for phenylalanine, leucine, isoleucine, and histidine were lower in the actual food product compared to the nutrient database. Previous work has also shown this to be true (Kafatos *et al*., 2000; Marshall & Judd, 1982; Porrini, Ciappellano, Simonetti, & Testolin, 1986).

Total Energy

The final calorie content in this product was around 306 kcal per 250 gram serving. Although this was 8% less that what was predicted, it still met the target value. 306 kcal provides 10 to 20% of daily energy needs for adults and over 30% for young children. On the other hand, although energy deficiencies remain prevalent in many areas of SSA, obesity and energy excess are becoming more common in SSA (FAO, 2017). A reason for this is due to increased intake of energy rich crops such as maize and cassava (FAO, 2017). Unfortunately, these foods lack many essential nutrients, including vitamin A. Thus, VAD is prevalent even in overweight and obese individuals (FAO, 2017). So, this food product is appropriate to serve as a single meal

replacement to provide adequate energy, but not in excess amounts to contribute to the problem of overnutrition.

Dietary Fat

The total fat content was tested to show that fat was available in the final product to allow for enhanced bioavailability of β-carotene. The final fat content of 7.4 grams/serving, can allow for increased bioavailability of β-carotene. Although the amount of fat needed to increase absorption varies among food source, as low as 2.4 grams of fat has been shown to sufficiently enhance the bioavailability of β-carotene in food (Ribaya-Mercado *et al.*, 2007). Additionally, 7.4 grams of fat is equivalent to 67 calories /serving, which is 22% of the total calories in this product. This percentage of fat is in line with macronutrient distribution ranges that recommend 20-30% of daily calorie needs come from fat. This shows that that this food product can be incorporated into a well-balanced meal plan.

Total Protein

The total protein content did not differ between the predicted and actual, containing 12 grams /serving. This provides 20 to 25% of daily protein needs for adults, around 60% for young children, and over 90% for infants. Consuming 100% of total daily protein needs in one serving is not recommended because the maximum anabolic utilization of protein at one time has been shown to be around 25 grams (Deutz & Wolfe, 2013). Any additional protein consumed at that time will be oxidized for energy or fat synthesis (Deutz & Wolfe, 2013). Rather, it is recommended that protein rich foods be incorporated throughout the day, which can be provided with a single serving of this product.

Essential Amino Acid Content

Since the body requires each essential amino acid in different ratios in comparison to one another to perform protein synthesis, and amino acid makeup of all food sources are different, the use of the amino acid scoring system developed by the Food and Nutrition Board/Institute of Medicines is often used to analyze protein quality (Institute of Medicine, 2005). In the final food product, all the essential amino acids, except methionine + cysteine, were present in amounts comparable to the reference amino acid scoring pattern.

Lysine is one of the most common limiting amino acids in SSA (Nuss, & Tanumihardjo, 2011). This is due to the high percentage of protein consumed from cereal grains, which are limiting in lysine (Nuss & Tanumihardjo, 2011). In many cereals, lysine is only present to meet between 56% and 86% of the requirement reference levels (Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, 2002), or around 30 mg/g protein (Young *et al*., 1994). Richer sources of lysine include animal products (85 mg/g protein) and legumes (65 mg/g protein) (Young *et al*., 1994).

In order to provide enough amounts of lysine in this product, two legumes, peanuts and chickpeas were combined. Peanuts are richer in total lysine, 640 mg/ 100 grams, compared to 480 mg/100 grams in chickpeas. However, the ratio of lysine per gram of protein is 69 in chickpeas and only 29 in peanuts (Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition, 2002). So, by incorporating peanuts which are high in lysine and total protein, with a secondary lysine rich source, the total lysine content was able to meet the reference amino acid scoring pattern.

Methionine and cysteine (not chemically analyzed) were the only amino acids that did not meet the reference amino acid scoring patterns. Sweet potatoes, peanuts, and chickpeas are all limited in these amino acids (Food and Agriculture Organization of the United Nations, 1970). The average amino acid score for these crops is only around 20 mg/g of protein, which is much lower than both animal products (37 mg/g) and maize (32 mg/g) (Food and Agriculture Organization of the United Nations, 1970; US Department of Agriculture, 1976-1986). However, even though methionine and cysteine were slightly less than the reference amino acid pattern, consumption of this product in addition to the cereal based foods that are already commonly consumed throughout the day in many of these target regions, the RDA of all essential amino acids can still be met.

Vitamin A

The vitamin A content in this final processed food product was significantly lower than the predicted value from the nutrient database. A large reason for this difference can be attributed to the large variation of β-carotene content in raw sweet potatoes. Hangenimana and others 1999a reported values as low as 111 µg/100 grams and up to 7,984 µg/100 grams fresh weight among varieties. Teow and others, 2007 also found great variations in light orange to dark orange varieties, reporting values from 2,980 µg/100 grams up to 22,600 µg/100 grams. Bengstsson *et al*. observed β-carotene content of 7 OFSP varieties and found β-carotene content to be between 3,732 µg to 9,648 µg/ 100 grams (Bengtsson, Namutebi, Alminger, & Svanberg, 2008). Very high β-carotene content has also been shown, with varieties containing 15,000 µg/100 grams (Lako and others 2007) to 28,100 µg/ 100 grams (Failla and others 2009).

The major differences in starting β-carotene content has been shown to be dependent on the color of the potato, growth conditions, harvesting times (peak being around late summer-early fall), and handling (Rodriguez-Amaya, 2000). The sweet potatoes that were used in this product were obtained in October, around peak harvesting times, from a local grocery store in Athens, Georgia. However, these potatoes were originally grown in Mississippi, and the actual harvesting period is unknown. Additionally, the growth conditions, storage, and post-harvest handling was not observed, which also could explain the low initial β-carotene content. The results of this work show that all conditions from planting to storage and handling can impact β-carotene content of raw sweet potatoes.

The use of multiple thermal processing steps also attributed to the lower β-carotene content in the final product. The raw sweet potatoes were initially steamed to produce a puree for the final product. Steaming was used to process the raw sweet potatoes because this process has shown higher retention rates compared to boiled, roasted, and dried sweet potatoes (Bechoff & Dhuique-Mayer, 2017; De Moura *et al.*, 2015). Work performed by Donado-Pestana and others, 2012 showed an average loss of 40% following drying and 19% loss following boiling, while steaming only resulted in a 10% loss of β-carotene. De Moura *et al*., 2015, also found drying to lose greater amounts of β-carotene, with an 80% reduction following drying and storage. The shorter cooking time required for steaming could explain the greater retention in steaming compared to other thermal processes (Van Jaarsveld, Harmse, Nestel, & Rodriguez-Amaya, 2006). Additionally, the use of steaming can actually increase the β-carotene content because of the greater availability of β-carotene inside of the food matrix following processing (Donado-Pestana, Salgado, de Oliveira Rios, dos Santos, & Jablonski, 2012; Azizah, Wee, Azizah, &

Azizah, 2009). Further work on this product that tests different thermal processes, including the use of blanching (shown to cause little to no loss of β-carotene), may be appropriate to retain greater amounts of β-carotene (Nascimento et al., 2007). Additionally, since further thermal sterilization processing in performed on this product, eliminating this initial processing step could be tested.

The second thermal processing step included high heat sterilization that resulted in a shelf stable product. There was a 60% retention in β-carotene following sterilization. This is similar to previous work performed on carrot puree, showing retention rates around 70% to 75% following sterilization (Rock *et al.*, 1998). The sterilized carrot puree also contained β-carotene isomers, like what was shown in this current work (Rock *et al.*, 1998). Similar to other thermal processes, varying the temperature and processing times of sterilization has shown to effect β-carotene retention (Rock *et al.*, 1998). So, because the thermal processing used in this current work resulted in sterilization much greater than a 12-log reduction of organisms (actual ~20 log reduction), a shorter processing time should be tested.

In addition to low starting β-carotene content and processing loss, β-carotene could have been lost as a result of degradation when exposed to light and oxygen. This degradation could also be an explanation for the high variability within the samples of the unsterilized and sterilized product (Table 20). The initial cooking and mixing steps of this food product were performed under normal light, which could have resulted in β-carotene loss. Additionally, oxidative damage could have resulted during the product development, packaging, and storage steps due to the lack of access to a nitrogen flush.

The loss of β-carotene in this work demonstrate the need for quality measures throughout all steps in the development and distribution of this product. The initial β-carotene content is one of the most variable conditions that can greatly impact the end β-carotene content of this product. Not only are there differences among variety, but the β-carotene content can differ within varieties depending on environmental conditions, seasonal changes, and harvesting periods (Rodriguez-Amaya, 2000). Proper storage, handling, and transportation methods to the processing facility are all post-harvesting steps that can cause further losses of β-carotene. Processing facilities should be equipped and educated on processes to limit degradation of β-carotene. This includes limiting exposure to light and air, the use of proper lighting, and vacuum sealing all packages. Minimizing thermal treatments and standardizing these processes are also essential to ensure consistency in the retention rate of β-carotene. Finally, β-carotene degradation can occur through long periods of storage of this product. Periodical β-carotene testing throughout all steps and standardization of all methods are needed so that the β-carotene content of this final product is consistent.

In the end, after both thermal processes, one serving of this final product did contain 5,893 ± 135 µg β-carotene and 491 ± 11 µg RAE. Although lower than expected, this result is similar to the final β-carotene content of 150 grams of processed sweet potato varieties, reporting 840 to 5,355 µg β-carotene (Kidmose and others 2007). Higher values of processed sweet potatoes have been reported with values at 10,950 to 37, 965 µg β-carotene / 150 grams (Bengtsson *et al*., 2008), but starting β-carotene content, handling, and storage are all conditions that could have resulted in these greater values. Additionally, even following this processing

loss, 491 µg RAE is still enough to meet the daily needs of young children and half for most other individuals.

An assumption that was made in this study was that with the addition of fat in this product, provided from the peanut butter, would allow for enhanced bioavailability of the β-carotene. This assumption was made from many previous studies showing that β-carotene with the addition of fat significantly increased absorption (Huo, Ferruzzi, Schwartz, & Failla, 2007; Mills *et al.*, 2008; Ribaya-Mercado *et al.*, 2007; Van Jaarsveld *et al.*, 2005). In addition, although thermal processing does cause some loss of β-carotene, it also breaks up the matrix of β-carotene in the crop, which has been shown to actually increase the bioavailability (Bechoff & Dhuique-Mayer, 2017). So, by including both a fat source and a thermal processing step to produce this product, the β-carotene that is retained in this product can be more readily absorbed and utilized. Further testing of the bioavailability of this product should be performed to validate this assumption.

Iron and Zinc Content

Two additional essential nutrients, iron and zinc, were also analyzed in this food product. These two nutrients are also commonly deficient among women of childbearing age and young children in SSA (Black *et al.*, 2008). Both nutrients are classified as trace minerals because the body only needs small amounts to perform biological functions. For iron, a good food source is considered one that supplies 1.8 to 3.4 mg per serving, which is similar to what was determined in this food product (FDA, 2018). Compared to RDA levels, the amount in this food product meets 18-29% of daily needs for males and 11-29% of daily needs for females (Institute of Medicine of the National Academies, 2005). This food product also contained small amounts of

zinc, providing ~10% of the daily needs of zinc for adults and close to 36% of daily needs for children (Institute of Medicine of the National Academies, 2005). Thus, this food product can assist in providing these nutrients in small amounts and meeting daily needs. Further supplementation of these nutrients would be needed to provide these nutrients in adequate amounts.

Overall, the results of the nutrient chemical analysis of this product shows that producing a single food product that meets the daily requirements of various essential nutrients is challenging without further direct supplementation. Combination dishes like this current work have also shown this to be true. Sweet potato flour has been combined with indigenous crops to produce high calorie and high protein infant weaning products (Mahmoud & Anany, 2014) and supplementary food products (Kunyanga, Imungi, Okoth, Vadivel, & Biesalski, 2012). However, similar to the results of this study, these products were rich in some nutrients, but were insufficient in other essential nutrients including lysine (Maumoud & Anay, 2014) and vitamin A (Kunyanga, Imungi, Okoth, Vadivel, & Biesalski, 2012). Furthermore, the loss of β-carotene in the drying process must also be considered in these products. A variety of other infant weaning and supplementary combination products have also been produced, but focus primarily on protein and calorie deficiencies, and are limited in many essential micronutrients (Ejigui, Savoie, Marin, & Desrosiers, 2007; Omueti, Jaiyeola, Otegbayo, Ajomale, & Afolabi, 2009).

Versatility

In addition to producing a nutrient rich product, the simple combination of regionally available ingredients makes this product very versatile. In this work, chickpeas were used as the complimentary protein source to peanuts because of the increased availability and consumption

in many regions of SSA, including Ethiopia and other Northern and Eastern countries of Africa (Nedumaran *et al*., 2015). However, there has also been an increase in production of additional legumes including cowpeas (western Africa), pigeon peas (eastern and southern regions), and soybeans (western Africa) (Nedumaran *et al*., 2015). Since these three legumes have similar nutrient content (Table 22), they can simply be substituted for one another in the final food product (Table 23). These legumes can be found either whole, processed into a flour, or as isolates (i.e. texturized soy protein (TSP)). The amino acid profile does not differ among forms, which can allow for each to be utilized in this product. However, because of the moisture content of each, the final weight in the product may need to be adjusted (Banaszkiewicz, 2011). The option to utilize various available legumes and in all different forms further allows this product to be both agriculturally and economically sustainable.

The availability of the ingredients and simplicity of the product formulation also allows for this product to be produced by individuals in the form of a recipe. A simple way of implementing this would be to produce a thermally processed sweet potato puree that contains the instructions to produce this combined product on the label. Among all three ingredients, raw sweet potatoes have the shortest shelf life, and will perish rapidly if not processed soon after harvest. Thus, by thermally processing the sweet potato alone, perishability of the sweet potato can be addressed more effectively, and problems of rancidity, that could be caused by the combined processed product, would not be of a concern. Additionally, individually packaging each ingredient would also address limitations in the transportation and availability that comes with central processing.

Table 22:
Nutrient Comparison of Chickpeas, Pigeon Peas, Cowpeas, and Soybeans per 100 Grams[a]

	Chickpeas	Pigeon Peas	Cowpeas	Soybeans
Energy (kcal)	139	121	116	172
Fat (g)	2.77	0.38	0.53	0.38
Protein (g)	7	7	8	18
Vitamin A (µg RAE)	1.15	0.15	0.75	0.45
Iron (mg)	1.07	1.11	2.51	5.14
Zinc (mg)	0.63	0.90	1.29	1.15
Histidine (mg)	0.20	0.24	0.24	0.45
Isoleucine (mg)	0.30	0.25	0.31	0.81
Leucine (mg)	0.51	0.48	0.59	1.36
Lysine (mg)	0.48	0.47	0.52	1.11
Methionine (mg)	0.09	0.08	0.11	0.22
Cysteine (mg)	0.10	0.08	0.09	0.27
Phenylalanine (mg)	0.38	0.58	0.45	0.87
Tyrosine (mg)	0.18	0.17	0.25	0.63
Threonine (mg)	0.26	0.24	0.29	0.72
Tryptophan (mg)	0.07	0.07	0.10	0.24
Valine (mg)	0.30	0.29	0.37	0.83

a. Nutrient values obtained from The Food Processor Nutrition and Fitness Software (2016)

Table 23:
Theoretical Nutrient Content of Final Formulation Substituting Chickpeas with Pigeon Peas, Cowpeas, and Soybeans[a]

	Final formulation with chickpeas	Final formulation with pigeon peas	Final formulation with cowpeas	Final formulation with soybeans
Energy (kcal)	334	319	314	363
Fat (g)	9	7	7	15
Protein (g)	12	12	13	22
Vitamin A (µg RAE)	1442	1442	1442	1442
Iron (mg)	2.19	2.22	3.45	3.45
Zinc (mg)	1.34	1.58	1.92	1.92
Histidine (mg)	0.29	0.33	0.33	0.52
Isoleucine (mg)	0.44	0.39	0.45	0.88
Leucine (mg)	0.80	0.78	0.88	1.54
Lysine (mg)	0.62	0.62	0.66	1.18
Methionine (mg)	0.17	0.15	0.18	0.28
Cysteine (mg)	0.15	0.14	0.14	0.30
Phenylalanine (mg)	0.64	0.82	0.71	1.07
Tyrosine (mg)	0.32	0.31	0.38	0.71
Threonine (mg)	0.45	0.43	0.48	0.85
Tryptophan (mg)	0.15	0.14	0.17	0.30
Valine (mg)	0.52	0.51	0.58	0.98

a. Nutrient values obtained from The Food Processor Nutrition and Fitness Software (2016)

CHAPTER 6

CONCLUSION

Overall, a single nutrient rich product was able to be produced from three regionally available crops to address both vitamin A and micronutrient deficiencies. The use of computer modeling allowed for the development of this product in a relatively easy manner. The database values for total energy, protein, and essential amino acids were all in line with what was chemically analyzed in the final product. Additionally, iron and zinc were also similar to the database values. However, the major downfall of computer modeling, as shown in this work with the low β-carotene content, is that there can be great variation in the nutrient values among varieties of crops. Initial β-carotene in sweet potatoes varies greatly between crop varieties, growing conditions, harvesting, storage, processing, and handling (Rodriguez-Amaya, 2000). As a result, nutrient databases can vastly overestimate, as was the case in this present study, as well as underestimate nutrient content. So, nutrient databases can serve as good estimates of the more heat and oxygen stable nutrients, but careful attention should be taken for nutrients such as β-carotene that can be easily destroyed.

The second part of this study looked at the retention of β-carotene following thermal sterilization. There was minimal but significant loss during the sterilization. However, there is limited data on the effects of β-carotene retention of sweet potatoes following sterilization processes (Rock *et al*., 1998; Marx *et al.*, 2003). Further work is needed to look at ways to produce a shelf stable product that also retains maximum amounts of β-carotene.

The final vitamin A content of this food product was lower than the predicted value but still provided 100% of the daily needs for young children, and half of the daily needs for adults. Additionally, the addition of fat can allow for the β-carotene that is in the product to be more bioavailable for individuals. So, by incorporating this dish into one or two meals per day can meet the daily vitamin A requirements, provide all essential amino acids, and provide appropriate amounts of energy to address multiple forms of undernutrition.

Limitations and Further Research

There are multiple limitations in this research. The first is the use of US grown sweet potatoes in place of OFSP varieties. There is significant variability of β-carotene content shown between varieties of sweet potatoes (Hotz *et al*., 2012). Additionally, the taste and texture of these varieties also differ, which could impact the final product (Hotz *et al*., 2012). Further work will be needed to test the nutrient content and physical properties of this product using the sweet potato varieties grown in SSA. Secondly, only one variety of sweet potato was used to produce this product, and it was assumed that the β-carotene content of the variety used was comparable to other US grown sweet potatoes. Another limitation is the assumption that the fat in this product improves bioavailability. Bioavailability testing should be performed to validate that the assumption that with the addition of fat, the bioavailability of β-carotene is increased. Whole chickpeas were used in this final product, but the use of chickpea flour could be tested to increase protein content or allow for the use of more sweet potatoes to be added to increase total vitamin A content. Different legumes can also be tested, including soybeans, which show a similar amino acid content of animal-based products (Young, 1991). Finally, the practicality of producing this product and consumer acceptance will also need to be tested in the future.

References

1. Amagloh, F. K., & Coad, J. (2014). Orange-fleshed sweet potato-based infant food is a better source of dietary vitamin A than a maize—Legume blend as complementary food. Food and nutrition bulletin, 35(1), 51-59.
2. Amorim-Carrilho, K. T., Cepeda, A., Fente, C., & Regal, P. (2014). Review of methods for analysis of carotenoids. TrAC Trends in Analytical Chemistry, 56, 49-73.
3. The American Diabetes Association and the American Dietetic Association. (1995). Exchange Lists for Meal Planning.
4. Azizah, A. H., Wee, K. C., Azizah, O., & Azizah, M. (2009). Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (Cucurbita moschato). International Food Research Journal, 16(1), 45-51.
5. Banaszkiewicz, T. (2011). Nutritional value of soybean meal. In Soybean and nutrition. IntechOpen.
6. Bechoff, A., & Dhuique-Mayer, C. (2017). Factors influencing micronutrient bioavailability in biofortified crops. Annals of the New York Academy of Sciences, 1390(1), 74-87.
7. Beijer, M. R., Kraal, G., & den Haan, J. M. M. (2014). Vitamin A and dendritic cell differentiation. Immunology, 142(1), 39–45. http://doi.org/10.1111/imm.12228.

8. Bengtsson, A., Namutebi, A., Alminger, M. L., & Svanberg, U. (2008). Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. Journal of food composition and analysis, 21(2), 134-143.

9. Black R.E., Allen L.H., Bhutta Z.A., Caulfield L.E., de Onis M., Ezzati M. *et al*. (2008) Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 371, 243–260.

10. Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., De Onis, M., ... & Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. The lancet, 382(9890), 427-451.

11. Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Global food security, 12, 49-58.

12. Bovell-Benjamin, A. C. (2007). Sweet potato: a review of its past, present, and future role in human nutrition. Advances in food and nutrition research, 52, 1-59.

13. Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., Eldridge, A. L., Schwartz, S. J. & White, W. S. (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. Am. J. Clin. Nutr. 80: 396–403.

14. Castenmiler, J.J.M., West, C. E., Linssen, J.P.H., van het Hof, K. H. & Voragen, A.G.J. (1999). The food matrix of spinach is a limiting factor in determining the bioavailability of β-carotene and to a lesser extent of lutein in humans. J. Nutr. 129:349–355.

15. Clagett-Dame, M., & Knutson, D. (2011). Vitamin A in Reproduction and Development. Nutrients, 3(4), 385–428. http://doi.org/10.3390/nu3040385.

16. Cohen, N., Rahman, H., Sprague, J., Jalil, M. A., Leemhuis de Regt, E., & Mitra, M. (1985). Prevalence and determinants of nutritional blindness in Bangladesh children. World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales, 38(3), 317-30.

17. Daryanto, S., Wang, L., & Jacinthe, P. A. (2015). Global synthesis of drought effects on cereal production. In AGU Fall Meeting Abstracts.

18. De Moura, F. F., Miloff, A., & Boy, E. (2015). Retention of provitamin A carotenoids in staple crops targeted for biofortification in Africa: cassava, maize and sweet potato. Critical reviews in food science and nutrition, 55(9), 1246-1269.

19. Dersch, H., & Zile, M. H. (1993). Induction of normal cardiovascular development in the vitamin A-deprived quail embryo by natural retinoids. Developmental biology, 160(2), 424-433.

20. Deutz, N. E., & Wolfe, R. R. (2013). Is there a maximal anabolic response to protein intake with a meal? Clinical Nutrition, 32(2), 309-313. doi:10.1016/j.clnu.2012.11.018.

21. Dewey, K. G., & Begum, K. (2011). Long-term consequences of stunting in early life. Maternal & child nutrition, 7, 5-18.

22. Diao, M. M., André, S., & Membré, J. M. (2014). Meta-analysis of D-values of proteolytic Clostridium botulinum and its surrogate strain Clostridium sporogenes PA 3679. International journal of food microbiology, 174, 23-30.

23. Dimitrov, N. V., Meyer, C., Ullrey, D. E., Chenoweth, W., Michelakis, A., Malone, W., ... & Fink, G. (1988). Bioavailability of beta-carotene in humans. The American journal of clinical nutrition, 48(2), 298-304.

24. Donado-Pestana, C. M., Salgado, J. M., de Oliveira Rios, A., dos Santos, P. R., & Jablonski, A. (2012). Stability of carotenoids, total phenolics and in vitro antioxidant capacity in the thermal processing of orange-fleshed sweet potato (Ipomoea batatas Lam.) cultivars grown in Brazil. Plant foods for human nutrition, 67(3), 262-270.

25. Duriancik, D. M., Lackey, D. E., & Hoag, K. A. (2010). Vitamin A as a regulator of antigen presenting cells. The Journal of nutrition, 140(8), 1395-1399.

26. Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T. (2007). Improvement of the nutritional quality of a traditional complementary porridge made of fermented yellow maize (Zea mays): Effect of maize–legume combinations and traditional processing methods. Food and nutrition bulletin, 28(1), 23-34.

27. Extract from 2014 Catalogue of Orange-fleshed sweetpotato for Africa (second edition) S. Tumwegamire, R.O.M. Mwanga, M.I. Andrade, J.W. Low, G.N. Ssemakula, S.M. Laurie, F.P. Chipungu, J. Ndirigue, S. Agili, L. Karanja, M.Chiona, J.C. Njoku, K. Mtunda, J. Ricardo, K. Adofo, E. Carey and W.J. Grüneberg. 2014. Orange-fleshed Sweetpotato for Africa. Catalogue 2014 (Second Edition). International Potato Center (CIP) Lima, Peru. 74p. ISBN: 978-92-9060-439-6 DOI: 10.4160/9789290604396.

28. Failla ML, Thakkar SK, Kim JY. (2009). In vitro bioaccessibility of beta-carotene in orange-fleshed sweet potato (Ipomoea batatas, Lam.). J Agric Food Chem 57:10922–27.

29. FAO. (1997). Part III. Disorders of malnutrition. Retrieved July 25, 2018, from http://www.fao.org/docrep/W0073e/w0073e05.htm#P2936_334096

30. FAO. (2007). The State of Food and Agriculture, 2007. Rome. 2007.

31. FAO. (2017). Regional Overview of Food Security and Nutrition in Africa 2017. The food security and nutrition–conflict nexus: building resilience for food security, nutrition and peace. Accra.

32. FAO. (2018). Food Security Indicators. Accessed: http://www.fao.org/economic/ess/ess-fs/ess-fadata/en/#.W9n_EflKiuV.

33. FAO, IFAD, UNICEF, WFP and WHO. (2018). The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO.

34. FAOSTAT. (2007). "FAOSTAT-Agriculture, Production, Crops." http://faostat.fao.org/.

35. FDA. (2018). CFR-Code of Federal Regulations Title 21. Updated on 1 April 2018. Accessed at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.54

36. Food and Agriculture Organization of the United Nations. (1970). Amino-acid content of foods and biological data on proteins. Nutr. Stud.# 24. Food Policy and Food Sci Serv., Nutr. Div.

37. Food and Agriculture Organization of the United Nations. (2000). The State of food insecurity in the world 2000. Rome, Italy.

38. Food and Nutrition Board, Institute of Medicine. Vitamin A. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese,

Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, D.C.: National Academy Press; 2001:65-126.

39. Gebretsadikan, T. M., Bultosa, G., Forsido, S. F., & Astatkie, T. (2015). Nutritional quality and acceptability of sweet potato–soybean–moringa composite porridge. Nutrition & Food Science, 45(6), 845-858.

40. Hagenimana V, Carey EE, Gichuki ST, Oyunga MA, Immungi JK. (1999a). Carotenoid contents in fresh, dried, and processed sweet potato products. Eco Food Nutr 37:455–73.

41. Hagenimana V, Low J, Anyanago M, Kurz K, Gichuki ST, Kabira J. (2001). Enhancing vitamin A intake in young children in western Kenya: orange-fleshed sweetpotatoes and women farmers can serve as key entry points. Food Nutr. Bull., 22: 376-387.

42. Hagenimana, V., Oyunga, M.A., Low, J., Njoroge, S.M., Gichuki, S., and Kabira, J. (1999b.). The effects of women farmers' adoption of orange-fleshed sweetpotatoes: Raising vitamin A intake in Kenya. ICRW/OMNI Research Program, Research Report Series 3. International Center for Research on Women, Washington, DC, 24p.

43. Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1821(1), 70-77.

44. HarvestChoice. Groundnut. Retrieved July 22, 2018, from http://harvestchoice.org/commodities/groundnut---

45. Haskell, M. J., Jamil, K. M., Hassan, F., Peerson, J. M., Hossain, M. I., Fuchs, G. J., & Brown, K. H. (2004). Daily consumption of Indian spinach (Basella alba) or sweet

potatoes has a positive effect on total-body vitamin A stores in Bangladeshi men. The American Journal of clinical nutrition, 80(3), 705-714.

46. Hotz, C., Loechl, C., de Brauw, A., Eozenou, P., Gilligan, D., Moursi, M., ... & Meenakshi, J. V. (2012). A large-scale intervention to introduce orange sweet potato in rural Mozambique increases vitamin A intakes among children and women. British Journal of Nutrition, 108(1), 163-176.

47. Huo, T., Ferruzzi, M. G., Schwartz, S. J., & Failla, M. L. (2007). Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. Journal of Agricultural and Food CHemistry, 55(22), 8950-8957

48. International Potato Center (2017). "Sweetpotato in Africa." Retrieved from: cipotato.org/research/sweetpotato-in-africa/.

49. Institute of Medicine of the National Academies (2005). Dietary Reference Intakes for Energy, Carbohydrate, Fiber Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. National Academies Press, Washington, DC, www.nap.edu.

50. Jalal, F., Nesheim, M. C., Agus, Z., Sanjur, D. & Habicht, J. P. (1998). Serum retinol concentrations in children are affected by food sources of β-carotene, fat intake, and athelminitc drug treatment. Am. J. Clin. Nutr. 68:623–629.

51. Janila, P., Variath, M. T., Pandey, M. K., Desmae, H., Motagi, B. N., Okori, P., … Varshney, R. K. (2016). Genomic Tools in Groundnut Breeding Program: Status and Perspectives. Frontiers in Plant Science, 7, 289. http://doi.org/10.3389/fpls.2016.00289

52. Jayarajan, P., Reddy, V. & Mohanram, M. (1980). Effect of dietary fat on absorption of β-carotene from green leafy vegetables in children. Indian J. Med. Res. 71:53–56.

53. Jensen, J., Rustad, P. I., Kolnes, A. J., & Lai, Y.-C. (2011). The Role of Skeletal Muscle Glycogen Breakdown for Regulation of Insulin Sensitivity by Exercise. Frontiers in Physiology, 2, 112. http://doi.org/10.3389/fphys.2011.00112.

54. Johansen, A. M. W., Lie, R. T., Wilcox, A. J., Andersen, L. F., & Drevon, C. A. (2008). Maternal dietary intake of vitamin A and risk of orofacial clefts: a population-based case-control study in Norway. American journal of epidemiology, 167(10), 1164-1170.

55. Joint FAO/WHO/UNU Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (2002 : Geneva, Switzerland), Food and Agriculture Organization of the United Nations, World Health Organization & United Nations University. (2007). Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. Geneva: World Health Organization. http://www.who.int/iris/handle/10665/43411.

56. Kafatos, A., Verhagen, H., Moschandreas, J., Apostolaki, I., & Van Westerop, J. J. (2000). Mediterranean diet of Crete: foods and nutrient content. Journal of the American Dietetic Association, 100(12), 1487-1493.

57. Kapinga, R., Tumwegamire, S., & Ndunguru, J. (2007). Status report of VITAA (Vitamin A for Africa): A partnership program combating Vitamin A deficiency through increased utilization of orange-fleshed sweetpotato in sub-Saharan Africa. *International Potato Centre (CIP)-VITAA, Uganda*, 37-38.

58. Kidmose U, Christensen LP, Agili SM, Thilsted SH. (2007). Effect of home preparation practices on the content of provitamin A carotenoids in coloured sweet potato varieties (Ipomoea batatas Lam.) from Kenya. Innov Food Sci Emerg Tech 8:399–406.

59. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. PubChem 2019 update: improved access to chemical data. Nucleic Acids Res. 2019 Jan 8; 47(D1):D1102-1109. doi:10.1093/nar/gky1033. [PubMed PMID: 30371825]

60. Kunyanga, C., Imungi, J., Okoth, M., Vadivel, V., & Biesalski, H. K. (2012). Development, acceptability, and nutritional characteristics of a low-cost, shelf-stable supplementary food product for vulnerable groups in Kenya. Food and nutrition bulletin, 33(1), 43-52.

61. LaComb RP, Taylor ML, Noble JM. (1992). Comparative evaluation of four microcomputer nutrient analysis software packages using 24-hour dietary recalls of homeless children. J Am Diet Assoc. 92:1391-1392.

62. Lako J, Trenerry VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R. (2007). Phytochemical flavonols, carotenoids, and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem 101:1727–41.

63. Laurie, S. M., Faber, M., & Claasen, N. (2018). Incorporating orange-fleshed sweet potato into the food system as a strategy for improved nutrition: The context of South Africa. Food Research International, 104, 77-85.

64. Laurie, S. M., & Van Heerden, S. M. (2012). Consumer acceptability of four products made from beta-carotene-rich sweet potato. African Journal of Food Science, 6(4), 96-103.

65. Laurie, S. M., Van Jaarsveld, P. J., Faber, M., Philpott, M. F., & Labuschagne, M. T. (2012). Trans-β-carotene, selected mineral content and potential nutritional contribution of 12 sweetpotato varieties. Journal of Food Composition and Analysis, 27(2), 151-159.

66. Linus Pauling Institute, Oregon State University. (2018). Carotenoids. Retrieved July 18, 2018, from http://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/carotenoids.

67. Linus Paulding institute, Oregon State University. (2015). Vitamin A. Retrieved October 16, 2018, from https://Ipi.oregonstate.edu/mic/vitamins/vitamin-A

68. Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F., & Tschirley, D. (2007). A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. The Journal of nutrition, 137(5), 1320-1327.

69. Low, J. W., Mwanga, R. O., Andrade, M., Carey, E., & Ball, A. M. (2017). Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. Global food security, 14, 23-30.

70. Low, J. W. (1997). Combating Vitamin A Deficiency Through the Use of Sweet Potato: Results from Phase I of an Action Research Project in South Nyanza, Kenya. International Potato Center.

71. Low, J. W., & van Jaarsveld, P. J. (2008). The potential contribution of bread buns fortified with β-carotene–rich sweet potato in Central Mozambique. Food and Nutrition Bulletin, 29(2), 98-107.

72. Maden, M. (2007). Retinoic acid in the development, regeneration and maintenance of the nervous system. Nature Reviews Neuroscience, 8(10), 755.

73. Maden, M., Gale, E., Kostetskii, I., & Zile, M. (1996). Vitamin A-deficient quail embryos have half a hindbrain and other neural defects. Current biology, 6(4), 417-426.

74. Mahmoud, A. H., & Anany, A. M. (2014). Nutritional and Sensory Evaluation of a complementary food formulated from rice, faba beans, sweet potato flour, and peanut oil. Food and Nutrition Bulletin, 25(4), 403-413.

75. Marshall, M. W., & Judd, J. T. (1982). Calculates vs. analyzed composition of four modified fat diets. Formulated to study effects in human subjects of kind and amount of dietary fat. *Journal of the American Dietetic Association*, *80*(6), 537-549.

76. Marx, M., Stuparic, M., Schieber, A., & Carle, R. (2003). Effects of thermal processing on trans–cis-isomerization of β-carotene in carrot juices and carotene-containing preparations. Food Chemistry, 83(4), 609-617.

77. Mark, M., Ghyselinck, N. B., & Chambon, P. (2009). Function of retinoic acid receptors during embryonic development. Nuclear receptor signaling, 7.

78. Mason, K. E. (1935). Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A-deficiency. American Journal of Anatomy, 57(2), 303-349.

79. Matyash, V., Liebisch, G., Kurzchalia, T. V., Shevchenko, A., & Schwudke, D. (2008). Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. Journal of lipid research, 49(5), 1137-1146.

80. McCullough, M. L., Karanja, N. M., Lin, P. H., Obarzanek, E. V. A., Phillips, K. M., Las, R. L., … & DASH Collaborative Research Group. (1999). Comparison of 4 nutrient databases with chemical composition data from the dietary approaches to stop hypertension trial. Journal of the American Dietetic Association, 99(8), S45-S53.

81. Menale, K., Bekele, S., & Geoffrey, M. (2010). Adoption and Impact of Improved Groundnut Varieties on Rural Poverty. Evidence from Rural (pp. 10-11). Uganda.

82. Mills, J. P., Tumuhimbise, G. A., Jamil, K. M., Thakkar, S. K., Failla, M. L., & Tanumihardjo, S. A. (2008). Sweet potato β-carotene bioefficacy is enhanced by dietary fat and not reduced by soluble fiber intake in Mongolian gerbils. The Journal of nutrition, 139(1), 44-50.

83. MOST, USAID Micronutrient Program. (2004). Cost Analysis of the National Vitamin A Supplementation Programs in Ghana, Nepal, and Zambia: A Synthesis of Three Studies. Arlington, Virginia, USA.

84. Mudege, N.N.; Mayanja, S.; Muzhingi, T. (2017). Women and men farmer perceptions of economic and health benefits of orange-fleshed sweet potato (OFSP) in Phalombe and Chikwawa districts in Malawi. Food Security. ISSN 1876-4517. 9(2): 387-400.

85. Müller, O., & Krawinkel, M. (2005). Malnutrition and health in developing countries. Canadian Medical Association Journal, 173(3), 279-286.

86. Nascimento, P., Fernandes, N. S., Mauro, M. A. and Kimura, M. (2007). Beta-carotene stability during drying and storage of cassava and sweet potato. In: Book of Abstract of the 2nd International Symposium on the Health Benefits of Fruits and Vegetables, Houston, USA. Acta Horticulturae, Wellington, NZ.

87. National Institute of Health Office of Dietary Supplements. (2010, March 03). Bioconversion of dietary provitamin A carotenoids to vitamin A in humans | The American Journal of Clinical Nutrition | Oxford Academic. Retrieved from https://academic.oup.com/ajcn/article/91/5/1468S/4597430.

88. Nedumaran, S., Abinaya, P., Jyosthnaa, P., Shraavya, B., Rao, P., & Bantilan, C. (2015). Grain Legumes Production, Consumption and Trade Trends in Developing Countries; Working Paper Series No. 60.

89. Nuss, E. T., & Tanumihardjo, S. A. (2011). Quality protein maize for Africa: closing the protein inadequacy gap in vulnerable populations. Advances in nutrition (Bethesda, Md.), 2(3), 217-24.

90. O'Byrne, S. M., & Blaner, W. S. (2013). Retinol and retinyl esters: biochemistry and physiology: Thematic Review Series: Fat-Soluble Vitamins: Vitamin A. Journal of Lipid Research, 54(7), 1731–1743. http://doi.org/10.1194/jlr.R037648.

91. O'Connell, Orla & Ryan, Lisa & O'Sullivan, Laurie & Aherne, Aisling & M O'Brien, Nora. (2008). Carotenoid Micellarization Varies Greatly Between Individual and Mixed Vegetables With or Without the Addition of Fat or Fiber. International journal for vitamin and nutrition research. Internationale Zeitschrift für Vitamin- und Ernährungsforschung. Journal international de vitaminologie et de nutrition. 78. 238-46. 10.1024/0300-9831.78.45.238.

92. OECD/FAS (2016), "Agriculture in Sub-Saharan Africa: Prospects and challenges for the next decade", in OECD-FAO Agricultural Outlook 2016-2025, OECD Publishing, Paris.

93. Oke, O. L., Redhead, J., & Hussain, M. A. (1990). Roots, tubers, plantains and bananas in human nutrition. FAO food and nutrition series, 24.

94. Omueti, O., Jaiyeola, O., Otegbayo, B., Ajomale, K., & Afolabi, O. (2009). Development and quality evaluation of low-cost, high-protein weaning food types: Prowena and

Propalm from soybean (Glycine max), groundnut (Arachis hypogea) and crayfish (Macrobrachium spp). British food journal, 111(2), 196-204.

95. Pénicaud, Caroline & Achir, Nawel & Dhuique-Mayer, Claudie & Dornier, Manuel & Bohuon, Philippe. (2011). Degradation of β-carotene during fruit and vegetable processing or storage: Reaction mechanisms and kinetic aspects: A review. Fruits. 66. 417-440. 10.1051/fruits/2011058.

96. Porrini, M., Ciappellano, S., Simonetti, P., & Testolin, G. (1986). Chemical composition of Italian cooked dishes. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin-und Ernahrungsforschung. Journal international de vitaminologie et de nutrition*, *56*(3), 263-268.

97. Ramaswamy, H. S., & Marcotte, M. (2006). Food processing: Principles and applications. Boca Raton: CRC Press.

98. Reboul, E. (2013). Absorption of Vitamin A and Carotenoids by the Enterocyte: Focus on Transport Proteins. Nutrients, 5(9), 3563–3581. http://doi.org/10.3390/nu5093563.

99. Ribaya-Mercado, J. D., Maramag, C. C., Tengco, L. W., Dolnikowski, G. G., Blumberg, J. B., & Solon, F. S. (2007). Carotene-rich plant foods ingested with minimal dietary fat enhance the total-body vitamin A pool size in Filipino schoolchildren as assessed by stable-isotope-dilution methodology–. The American journal of clinical nutrition, 85(4), 1041-1049.

100. Rock, C. L., Lovalvo, J. L., Emenhiser, C., Ruffin, M. T., Flatt, S. W., & Schwartz, S. J. (1998). Bioavailability of β-carotene is lower in raw than in processed carrots and spinach in women. The Journal of nutrition, 128(5), 913-916.

101. Rodriguez-Amaya, D. B. (2000). Some considerations in generating carotenoid data for food composition tables. Journal of food composition and analysis, 13(4), 641-647.).

102. Ross, A. C. (2012). Vitamin A and retinoic acid in T cell–related immunity. The American Journal of Clinical Nutrition, 96(5), 1166S–1172S. http://doi.org/10.3945/ajcn.112.034637.

103. Sauberlich, H. E., Hodges, R. E., Wallace, D. L., Kolder, H., Canham, J. E., Hood, J., Raica, W., Jr. & Lowry, L. K. (1974) Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam. Horm. 32:251–275.

104. Sayre, R., Beeching, J. R., Cahoon, E. B., Egesi, C., Fauquet, C., Fellman, J., … & Maziya-Dixon, B. (2011). The BioCassava plus program: biofortification of cassava for sub-Saharan Africa. Annual review of plant biology, 62, 251-272.).

105. Schönfeldt, H. C. and N. G. Hall (2012). "Dietary protein quality and malnutrition in Africa." British Journal of Nutrition 108(S2): S69-S76.

106. Seljak, B. K., Stibilj, V., Pograjc, L., Mis, N. F., & Benedik, E. (2013). Food composition databases for effective quality nutritional care. Food chemistry, 140(3), 553-561.

107. Semba, R. D. (1998). The role of vitamin A and related retinoids in immune function. Nutrition reviews, 56(1), S38-S48.

108. Shete, V., & Quadro, L. (2013). Mammalian metabolism of β-Carotene: gaps in knowledge. Nutrients, 5(12), 4849-4868. doi:10.3390/nu5124849.

109. Schönfeldt, H. C. and N. G. Hall (2012). "Dietary protein quality and malnutrition in Africa." British Journal of Nutrition 108(S2): S69-S76.

110. Sindi, K., Kiria, C., Low, J. W., Sopo, O., & Abidin, P. E. (2013). Rooting out hunger in Malawi with nutritious orange-fleshed sweetpotato: A baseline survey report, Blantyre, Malawi.

111. Smith, S. M., Dickman, E. D., Power, S. C., & Lancman, J. (1998). Retinoids and their receptors in vertebrate embryogenesis. The Journal of nutrition, 128(2), 467S-470S.

112. Snapp, S., Rahmanian, M., Batello, C. (2018) Pulse crops for sustainable farms in sub-Saharan Africa, edited by T. Calles. Rome, FAO.

113. Sommer, A., West, K. P., & Ross, A. C. (1996). Vitamin A deficiency: health, survival, and vision. Oxford University Press, USA.

114. Stephensen, C. B. (2001). "VITAMIN A, INFECTION, AND IMMUNE FUNCTION." Annual Review of Nutrition 21(1): 167-192.

115. Stephenson, K., *et al*. (2010). "Consuming cassava as a staple food places children 2-5 years old at risk for inadequate protein intake, an observational study in Kenya and Nigeria." Nutrition journal 9(1): 9.

116. Stevens, G. A., Bennett, J. E., Hennocq, Q., Lu, Y., De-Regil, L. M., Rogers, L., ... & Oehrle, S. P. (2015). Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. The Lancet Global Health, 3(9), e528-e536.

117. Tang, G., Gu, X. F., Hu, S. M., Xu, Q. M., Qin, J., Dolnikowski, G. G., ... & Yin, S. A. (1999). Green and yellow vegetables can maintain body stores of vitamin A in Chinese children–. The American journal of clinical nutrition, 70(6), 1069-1076.

118. Tawanda, M., Kyung-Jin, Y., Bermudez, O., Guangwen, T., & Hlanganiso Siwela, A. (2017). Peanut butter increases the bioavailability and bioconversion of kale p-carotene to vitamin A. Asia Pacific Journal of Clinical Nutrition, 26(6), 1039-1047. doi:10.6133/apjcn.112016.03.

119. Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV, Yencho GC. (2007). Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem 103:829–38.

120. Thurnham, D. I. (2007). Bioequivalence of β-carotene and retinol. Journal of the Science of Food and Agriculture, 87(1), 13-39.

121. Tseng, S. C., Hatchell, D. I. A. N. E., Tierney, N., Huang, A. J., & Sun, T. T. (1984). Expression of specific keratin markers by rabbit corneal, conjunctival, and esophageal epithelia during vitamin A deficiency. The Journal of cell biology, 99(6), 2279-2286.

122. Tumuhimbise, G. A., Namutebi, A., & Muyonga, J. H. (2009). Microstructure and in vitro beta carotene bioaccessibility of heat processed orange fleshed sweet potato. Plant foods for human nutrition, 64(4), 312.

123. Unicef. (2013). Improving child nutrition: the achievable imperative for global progress. New York: UNICEF, 1-114.

124. UNICEF EASTERN AND SOUTHERN AFRICA REGIONAL OFFICE. (2016). UNICEF Eastern and Southern Africa Regional Office Annual Report 2015. Accessed: https://www.unicef.org/about/annualreport/files/ESARO_2015_ROAR.pdf.

125. United Nations. (2015). The Millennium Development Goals Report (Rep.) (C. Way, Ed.). New York.

126. The United Nations Children's Fund (UNICEF). (2007). "Vitamin A Supplementation: A Decade of Progress."

127. United Nations Children's Fund. (2015). UNICEF's approach to scaling up nutrition for mothers and their children. Discussion paper. Programme Division, UNICEF, New York.

128. United States Department of Agriculture-Agricultural Research Service. (2019). USDA Food Composition Databases. Accessed at: https://ndb.nal.usda.gov/ndb/search/list.

129. USDA. (2019). Food Composition Databases Show Foods List. Retrieved August 29, 2018, from https://ndb.nal.usda.gov/ndb/search/list?qlookup=16087.

130. US Department of Agriculture. Agricultural handbook no. 8. Washington, DC: Agriculture Research Service, United States Department of Agriculture, 1976-1986.

131. van Berkum, S., Achterbosch, T., Linderhof, V., Godeschalk, F., & Vroege, W. (2017). *Dynamics of food systems in Sub-Saharan Africa* (No. 2017-072). Wageningen Economic Research.

132. van Greevenbroek, M.M.J., van Meer, G., Erkelens, D. W. & de Bruin, T.W.A. (1996). Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells. Atherosclerosis 121: 139–150.

133. van het Hof, K. H., Tijburg, L.B.M., Pietrzik, K. & Weststrate, J. A. (1999). Bioavailability of carotenoids and folate from different vegetables. Effect of disruption of the vegetable matrix. Br. J. Nutr. 82:203–212

134. van het Hof, K. H., West, C. E., Weststrate, J. A., & Hautvast, J. G. (2000). Dietary factors that affect the bioavailability of carotenoids. The Journal of nutrition, 130(3), 503-506

135. van Jaarsveld, P. J., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., & Benadé, A. J. S. (2005). β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test–. The American journal of clinical nutrition, 81(5), 1080-1087.

136. Van Jaarsveld, P. J., Harmse, E., Nestel, P., & Rodriguez-Amaya, D. B. (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. Journal of Food Composition and Analysis, 19(4), 321-329.

137. Vasilopoulou, E., Georga, K., Grilli, E., Linardou, A., Vithoulka, M., & Trichopoulou, A. (2003). COmpatibility of computed and chemically determined macronutrients and energy content of traditional Greek recipes. Journal of food composition and analysis, 16(6), 707-719.

138. von Doering, W., Sotiriou-Leventis, C., & Roth, W. R. (1995). Thermal interconversions among 15-cis-, 13-cis-, and all-trans-. beta.-carotene: kinetics, Arrhenius parameters, thermochemistry, and potential relevance to anticarcinogenicity of all-trans-. beta.-carotene. Journal of the American Chemical Society, 117(10), 2747-2757.

139. Washington, DC: Agriculture Research Service, United States Department of Agriculture, 1976 1994.

140. White, J. C., Highland, M., & Clagett-Dame, M. (2000). Abnormal development of the sinuatrial venous valve and posterior hindbrain may contribute to late fetal resorption of vitamin A-deficient rat embryos. Teratology, 62(6), 374-384.

141. WHO/FAO/UNU Expert Consultation. (2007). Protein and Amino Acid Requirements in Human Nutrition. World Health Organization, Geneva Report of a Joint

WHO/FAO/UNU Expert Consultation. Word Health Organization Technical Report No. 935.

142. World Health Organization. (2019). Database on vitamin A deficiency http://www.who.int/entity/vmnis/vitamina/data/en/.

143. Young, V. R. (1991). Soy protein in relation to human protein and amino acid nutrition. Journal of the American Dietetic Association, 91(7), 828+. Retrieved from http://link.galegroup.com.proxy-remote.galib.uga.edu/apps/doc/A11120322/AONE?u=uga&sid=AONE&xid=574ba775.

144. Young, V. R., & Pellett, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. The American journal of clinical nutrition, 59(5), 1203S-1212S.

145. Yunusa, I., Ibrahim, M. A., Ahmad, I. M., Kabir, N., Gidado, Z. M., Rabiu, Z., ... & Ezeanyika, L. (2015). U. S. Research Article Vitamin A Status for Sustainable Development.