ABSTRACT

FENG, SOFIA. Current Research Advances in Micronutrient Fortification on Sweet Potato Flour and Development of an Affordable Nutrition Product. (Under the direction of Dr. Jonathan C. Allen).

Sweet potatoes are a versatile crop. The low need for input of nutrients in the soil allows them to be considered a food security crop. They are also ranked among vegetables with the highest in nutritional value available in the United States and worldwide; they are known to be rich in nutrients that include carbohydrates, protein, fiber, beta-carotene, vitamin C, E and anthocyanins and contain significant iron and zinc. Sweet potato flour (SPF) can be derived from this nutrient-rich crop using simple processing methods. SPF is a nutrient-rich, shelf-stable product that can be further improved with nutrient fortification. SPF was prepared in a pilot plant and then analyzed for several important nutrients. The content of vitamins B_1, B_2, and B_3 and folic acid was determined with microbiological RIDA®SOFT vitamin assay kits. An ELISA test confirmed the folic acid quantity. Atomic absorption was used to quantify iron. The data obtained show that micronutrient levels comparable to Enriched Wheat Flour can be obtained in SPF if only niacin, iron and folic acid are added. The second phase of this project included design of a prototype product that incorporated sweet potato flour as the base for instant noodles. These noodles were made by blending SPF with soy and mung bean flours and xanthan gum (as a cohesive ingredient) and can potentially provide the base for a malnutrition-preventing food to be produced locally by relief agencies, especially in regions where these ingredients are locally available.

The noodles were produced and tested in an accelerated shelf life study to evaluate chemical changes that relate to nutritional variables. The noodles were stored up to 5 weeks

at 28, 35, 45 and 55 °C. Statistical analysis showed decreases in protein, lysine, water activity, moisture content, compressive load and β-carotene. Fat content, delta energy, compressive extension and measurement of L, A, and B color remained stable over time. This study aimed to (1) Formulate a similar standard of identity for SPF comparable to wheat flour with reference to vitamins B_1, B_2, B_3, folate and iron and (2) To develop a new malnutrition-preventing food product using ingredients commonly grown in some tropical countries for use by relief agencies in disaster situations as a way to improve their food insecurity status.

Current Research Advances in Micronutrient Fortification on Sweet Potato Flour and Development of an Affordable Nutrition Product

by
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DEDICATION

To my family and advisor who made my journey through graduate school possible.

BIOGRAPHY

My name is Sofia Feng and I am originally from Costa Rica. I arrived in Raleigh, NC on a Fulbright fellowship to pursue a Masters in 2011. Although my undergraduate degree was in Nutrition and I do not have a Food Science background, I feel content and confident by learning each day with where I am and what I do; science has always had my heart. My grad school application was managed through my scholarship to different schools and I was convinced I was going to Purdue University. However, NC State, through my current advisor, was the first one to respond to my admission, and after some research, I realized how strong the FBNS department was. I also preferred NC State for its location and weather. Originally, I thought I wanted to combine Nutrition with community work. However, I realized that Food Science was a better fit for me after taking a few food science classes. In my spare time I enjoy learning about other cultures. I also enjoy being a group fitness instructor and teaching for faculty, staff and students at the NC State Rec Center.

I like attending class and am appreciative of the high quality education NC State professors have to offer. I admire the level of scientific research, innovation and outreach, especially from the FBNS department. I also consider my participation at seminars and symposiums a remarkable part of my journey as a graduate student. I have been able to attend the Summer Institute on Global Food Security, 2012 at Purdue, both as a participant and speaker. I also attended the Global Food Security Seminar for Fulbrighters hosted at NC State in 2012, IFT at Chicago in 2013, the Graduate Student Research Symposium and IFT, in New Orleans.

I am now intending to obtain a PhD in Food Science. When I finish, I will pursue a job as a scientist in the food industry, trying to address local and global challenges in food insecurity, food safety, and nutrition. I have thoroughly enjoyed my journey as a graduate student thanks to my current field of study, my advisor, and the people I have connected with at FBNS. Although I have an undergraduate degree from another university, I consider NC State my Alma Mater. NC State has provided me with the tools to broaden my knowledge and I look forward to further developing my skills here.

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CHAPTER 1

LITERATURE REVIEW

Introduction

There are two classes of nutrients that are essential for human growth and life sustainment: macronutrients and micronutrients. Malnutrition is a condition in which the body does not absorb enough nutrients for its adequate development (Tulchinsky, 2010). Food insecurity is defined as a combination of circumstances that do not allow an adequate and sustained procurement of food. According to the Food and Agriculture Organization (2012), food insecurity affected around one billion people worldwide, with major clusters in Asia, Africa, Latin America, and the Caribbean. The World Health Organization (WHO, 2006) believes that more than 2 billion people worldwide suffer from vitamin and mineral deficiencies, primarily iodine, iron, vitamin A and zinc, with important health consequences. These deficiencies occur mostly in developing countries in which there is no easy availability of, or access to, foods that naturally contain the essential micronutrients. In developed countries, the rise of restrictive, vegan, and vegetarian diets has caused an increased incidence in vitamin and mineral deficiencies, another cause of malnutrition.

Macronutrient malnutrition occurs due to an imbalance of major macronutrients such as carbohydrates, fats and proteins. In early stages of malnutrition, muscle mass tends to be protected, while oxidation of glycogen and visceral fat takes place, However, as glucose and glycogen stores become depleted, muscle catabolism starts in order to meet metabolic needs (Byrd-Bredbenner, 2009). As a chronic condition that occurs with total energy deprivation, this clinical stage is called marasmus. A similar malnutrition condition is kwashiorkor, which occurs with protein deficient diets that may be adequate in total energy (Jaya Rao, 1974).

1.1 Micronutrient Malnutrition

Malnutrition can also occur due the restriction of a single or in a combination of specific micronutrients (i.e., vitamins and minerals). The micronutrient malnutrition that affects people worldwide can be responsible for many chronic diseases, such as osteoporosis and osteomalacia, thyroid deficiency, colorectal cancer and cardiovascular diseases (Tulchinsky, 2010).

There are more than 20 vitamins and minerals that are essential for humans. Deficiencies in folate, iodine, iron, vitamin A and zinc, in particular, have a significant impact on public health in both developing and industrialized countries. Folate (vitamin B-9), iodine, iron, vitamin A and zinc are grouped-in and called the deficient five (Tarver, 2013). Along with these micronutrients, vitamin B deficiencies are prevalent in many developing countries. Thiamin, riboflavin, and niacin can be deficient in diets that are low in animal products, fruits and vegetables, and where cereals are milled prior to consumption. The best solution for many populations might be fortification of the foods that are available (Kuczora & Richardson, 2012).

To combat these deficiencies, most industrialized countries and some developing nations have strong fortification and enrichment programs. Since 1998, 50 countries have implemented mandatory fortification programs for certain foods (Hermann, 2011). For example: salt is fortified with iodine, sugar and milk are both fortified with vitamins A (Arroyave, 2010), milk and milk products with vitamin D, while cereals and flours are enriched with thiamin, niacin, riboflavin, folic acid, and iron. These programs aim to restore the nutrients lost during food processing and have helped to eliminate some disorders caused by micronutrient deficiencies, such as goiter and neural tube defects (Tarver, 2013). Currently, about 70% of the households in industrialized and developing countries consume iodized salt (United Nations-Standing Committee on Nutrition, 2010). Despite the implementation of these programs, micronutrient malnutrition is still a problem. Bioavailability of fortified products, and the quality and quantities of fortificants that should be added to foods are still challenges to be studied (Tarver, 2013). The following sections discuss micronutrients that may be added to fortified foods.

1.1.1 Folate

Folate is the natural form of vitamin B-9. This terms account for all derivatives of pteroic acid that demonstrate vitamin activity in humans (Ball, 1994). Folate is a water-soluble vitamin that is an essential biochemical constituent of living cells, so most foods contain some. However, foods such as asparagus, dark green leafy vegetables, fruits and fruit juices, meat products, liver, eggs and fish are considered among the best sources of folate (Frankea, et al. 2008). Generally in nature, folates are bound to proteins and they are also bound to starch or storage polysaccharides. 5,6,7,8-Tetrahydrofolate (THF) and 7,8-dihydrofolate (DHF) are reduced forms of pteroylglutamic acid (PGA) that act as cofactor in biochemical reactions involving the mobilization of one-carbon units in intermediary metabolism. These reactions are essential for the synthesis of DNA base constituents (purines and pyridines) .

These reactions also important in the metabolism of amino acids, especially methionine (Ball, 1994).

Folate is characterized by three constituents: 1. A *p*-aminobenzoic molecule linked to 2. a pteridine ring and 3. one molecule of glutamic acid. Folate functions as a coenzyme or cosubstrate in single-carbon transfers in the synthesis of nucleic acids (DNA and RNA) and metabolism of amino acids (NIH, 2013).

“5-methyltetrahydrofolate (MTHF) is the physiologically active form of folate. When the methyl group from 5-MTHF is transferred to homocysteine, methionine is formed, from which the universal methyl group donor S-adenosyl-methionine (SAM) develops. 5,10-Methylene-THF is required for the synthesis of thymidine and 10-formyl-THF for the synthesis of purine” (Lucock, 2000). THFA is then responsible for the synthesis of methionine from homocysteine.

Folate deficiency as well as an excess of folic acid may impair DNA stability, gene expression, and cell proliferation (Hermann and Obeid, 2011). For example, in animal studies, high dosages of folic acid prevented the development of new tumors, but supported the progression of pre-existing tumors (Lucock, 2000). Periconceptional folic acid supplementation lowers the incidence of neural tube defects by as much as 20% to 60%, and helps produce and maintain new cells (Arroyave, 2010). According to U.S. Department of Agriculture, Agricultural Research Service (2012) cited in The National Institute of Health: “Spinach, liver, yeast, asparagus, and Brussels sprouts are among the foods with the highest levels of folate” (NIH, 2013). Folic acid plays a fundamental role in cell division and differentiation (Hermann and Obeid, 2011). However, folic acid, is a more stable form of folate. Folic acid is the synthetic form, which is added to many products such as breakfast cereals, breads, flours, pastas, cornmeal and white rice. In general products made from refined grains are fortified with folic acid.

1.1.2 Iodine

Iodide is an essential trace mineral. It is present naturally in some foods, added to others, and it is also available as a dietary supplement. Good sources of iodine include seaweed and seafood, dairy products, particularly milk, grain products, and eggs. Most fruits and vegetables contain iodine; however, the quantity depends of the type of soil, fertilizer use and irrigation practices (Institute of Medicine, 2001). Iodine is vital in human growth and development for its role in synthesis of thyroxin. Dietary iodine intake at proper levels is required for the synthesis of regulatory hormone thyroxine and inhibits the formation of a goiter (enlarged thyroid gland) that results from high production of TSH (thyroid stimulating hormone) (NIH, 2011).

Latin America's iodine consumption has improved as use of iodine-fortified salt has increased. The percentage of iodine deficiency, manifested as goiter cases, has declined (de Benoist, 2008). Currently, about 70% of the households in industrialized and developing countries consume iodized salt (United Nations-Standing Committee on Nutrition, 2010). By 2007, in South America and Central America the coverage of iodized salt was higher, with 92% and 81% consumption respectively, compared to the Caribbean where only 48% of the households were covered. The prevalence of goiter in the Latin America and the Caribbean (LAC) region is 13.3% with approximately 57 million cases (United Nations- Standing Committee on Nutrition, 2010)

1.1.3 Iron

Iron is the trace element present in the body at the highest concentration and it is also one of the most abundant metals in the planet (Byrd-Bredbenner, 2009). Daily supply of iron in the diet can be obtained from two iron sources: heme and non- heme iron. In plants, iron is an important catalytic component of the heme enzymes involved in oxygen transport as well as electron transfer. Important examples of these enzymes are: hemoglobin, cytochrome oxidase and catalase (Whitford, 2005).

According to the United Nations-Standing Committee on Nutrition (2010), iron deficiency is among the most prevalent of all micronutrient deficiencies in the LAC region. Iron deficiency anemia continues to be the most significant nutrient deficiency worldwide. Prevalence of iron deficiency anemia in Central America was 20.3%, 23.8% and 23.7% among non-pregnant women, pregnant women and children under five years of age, respectively. Similarly, high rates are prevalent among populations in South America (26.1%, 22.4% and 41.7%) and the Caribbean (33.3%, 36.8% and 33.5%) among same groups, respectively (United Nations-Standing Committee on Nutrition, 2010).

1.1.4 Vitamin A

Retinoids including retinol, retinal, retinoic acid, retinyl esters and certain carotenoids are forms of vitamin A, a fat-soluble vitamin (Arroyave, 2010). Vitamin A can be consumed in the diet in two forms: as preformed vitamin A or provitamin A carotenoids. Preformed vitamin A is abundant in foods from animal sources. The sources include dairy, fish, and meat. Provitamin A in the form of alpha-carotene, beta-carotene and beta-cryptoxanthin is found in green, orange, and yellow vegetables and fruits such as apricots, broccoli, cantaloupe, carrots, dark leafy greens, squash, sweet potatoes, and tomatoes. The human body converts these plant pigments into vitamin A. The deficiencies in industrialized countries are very rare because of the variety in the diet and multiple food sources that are vitamin A-fortified; however, in LAC region its prevalence is 11.5%; the number of cases is decreasing due to food fortification policies, particularly fortification of sugar (Arroyave, 2010). Vitamin A functions in reproduction and fetal development, normal vision, cell differentiation and growth, wound healing and immunity against infections, and as an antioxidant. Vitamin A is also known as the anti-infective vitamin (Tarver, 2013).

1.1.5 Zinc

Zinc is a trace mineral that is naturally present in a wide variety of foods such as red meats, poultry, beans, nuts and certain types of seafood (such as oysters, crab and lobster), whole grains, fortified breakfast cereals, and dairy products (Eitenmiller, et al., 2008). Its bioavailability is higher in animal foods than in plant foods because of the presence of phytates, which are also present in whole grains, cereals, legumes, and other foods, that inhibit zinc's absorption. The abundant presence of zinc in the body allows this mineral to be indispensable at the role in numerous cellular functions. It is involved in multiple metabolic processes, for example: DNA synthesis and gene expression, cell division, protein synthesis, and the catalytic activity of more than 300 enzymes (Tarver, 2013). Physiological action in the human body allows zinc to be required in blood clotting, normal development during gestation, infancy, and childhood, and proper vision, smell, taste, and immune function. Zinc deficiency causes arrested development in infants and children and delayed sexual development in adolescents. Hair loss, eye and skin sores, diarrhea and inadequate wound healing, are also symptoms of zinc deficiency (Tarver, 2013).

1.1.5 Thiamin

Thiamin is also known as Vitamin B_1. It is found in a wide variety of foods that include vegetable sources such as: sunflower seeds, legumes (navy beans, green peas), cereals (wheat germ, rice), asparagus, peanuts and mushrooms. Other sources are meats such as pork, beef and fish and organ meats, such as liver (Byrd-Bredbenner, 2009; Eitenmiller, et al 2008; Tarver, 2013). "Thiamin consists of a central carbon attached to a 6-member nitrogen containing ring and a 5-member sulfur containing ring." (NCBI, n.d.) In living tissues, thiamin is ubiquitous and can be found as thiamin pyrophosphate (TPP). TPP acts as a co-enzyme; usually working as a non-covalent binding agent for the apoenzyme (Ball, 1994). This protein binding allows the thiamin content in foods to be protected, to some extent. TPP that is not bound is destroyed in acidic conditions and it is sensitive to very high temperatures (over 95-100^{o}C). As the pH of the environment increases, thiamin becomes more susceptible to activation by heat (Ball, 1994).

The importance of thiamin fortification is demonstrated by its ability to mediate the effects of the disease beriberi in Asian countries where white rice was the staple food and preferred over brown rice because it stayed fresh for longer. The processing of the white rice into a highly polished grain involves the removal of fat contained in the germ along with thiamin. The result of the vitamin B-1 deficiency, beriberi, includes peripheral neuropathy and weakness, muscle pain, and enlargement of the heart leading into congestive heart failure. The symptoms of beriberi develop after only 14 days on a thiamin-free diet (Byrd-Bredbenner, 2009).

1.1.6 Riboflavin

Riboflavin is also known as Vitamin B_2. The name comes from its color. Flavin means yellow in Latin. It has a distinctive yellow-green fluorescence. Riboflavin contains 3 linked 6 membered rings with a sugar alcohol attached to the middle ring. Exposure to light causes riboflavin to break down (Byrd-Bredbenner, 2009). Milk products are naturally high in riboflavin as are other animal sources such as organ meats, for example, liver, and vegetable sources, for example, green beans, broccoli, and mushrooms.

Packaging of riboflavin for enriched and fortified foods, such as milk and flour, is important in order to prevent the light-induced breakdown. Paper and dark plastic containers are preferred over glass (Eitenmiller, et al., 2008).

Riboflavin is involved in metabolic reactions, especially as flavin co-enzymes with oxidation-reduction functions: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The reduced form of FAD, $FADH_2$, for example, is a participant in the electron transport chain in the citric acid cycle, which is part of the energy metabolism (Byrd-Bredbenner, 2009). The formation of niacin from the amino acid tryptophan requires FAD.

Riboflavin deficiency is known as ariboflavinosis and affects primarily the mouth, skin and red blood cells. Ariboflavinosis develops after 2 months on a riboflavin-deficient diet and it can be caused by either dietary deficiency or malabsorption disorders. Deficiencies of riboflavin are usually present along with other vitamin deficiencies because riboflavin is needed for their metabolic pathways (Byrd-Bredbenner, 2009).

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1.1.7 Niacin

Niacin is also known as Vitamin B-3 and it exists in two forms: nicotinic acid (niacin) and niacinamide. Niacin co-enzymes are Nicotine Adenine Dinucleotide (NAD) and Nicotine Adenine Dinucleotide Phosphate ($NADP^+$) (Eitenmiller, et al., 2008). These niacin co-enzymes participate in around 200 reactions in cellular metabolic pathways, especially the ones that produce ATP. Because almost every metabolic pathway uses these two co-enzymes, niacin deficiency causes a disease called pellagra, which comprehensively includes: dermatitis, diarrhea and dementia.

Niacin can be obtained from foods as the vitamin form or it can be synthesized from the essential amino acid tryptophan. “Poultry, meat and fish provide 25% of the preformed niacin and another 11% comes from enriched bread and other enriched products.” (Byrd-Bredbenner, 2009).

1.2 Strategies to Combat Malnutrition

Public health initiatives to combat micronutrient deficiencies include supplementation and fortification strategies; however, these efforts are limited in most developing countries due to government budget constraints, public health policies and lack of access or variety to the fortified products. A more recent initiative involves biofortification and in extreme acute malnutrition, food aid programs (Tarver, 2013).

1.2.1. Supplementation

Clinical supplementation in acute malnutrition consists in a type of therapy at clinical level with prescription of sources when normal levels cannot be restored within an acceptable timeframe. Despite the benefits, dietary supplementation has limitations. Supplements are only provided by healthcare professionals as an extreme public health measure in order to combat malnutrition. In developing nations, over-the-counter supplementation is a significant constraint. Determinant factors such as low income, education level, community infrastructure, age and household size conditions impact micronutrient supplementation and accessibility (Marini, 2003). Iron supplements tend to cause abdominal discomfort and hardness of the stools; because of this iron-deficiency anemia patients tend not to consume this. Other independent factors such as migration, inadequate program support, and inadequate delivery system could negatively impact treatment compliance and thus, the intended effect of the supplementation. (Galloway & Mcguire, 1994)

1.2.2. Fortification

The World Health Organization (WHO), defines food fortification as the practice of deliberately increasing the content of an essential micronutrient, i.e., vitamins and minerals (including trace elements) in a food, in order to improve the nutritional quality of the food supply and provide a public health benefit with minimal health risk. Deficiencies in some groups of people at special risk require supplementation, but the most effective way to meet community health needs safely is by population-based approaches involving food fortification (Tulchinsky, 2010).

"Fortification is the process of adding micronutrients (vitamins and minerals) to foods in which they are absent, present only in small quantities or when food processing significantly reduces the natural micronutrient content of the food" (Kuczora & Richardson, 2012). Enrichment, different from fortification, as it is a strategy in which only the nutrients lost during the process are restored. In practice, fortification goes above these enrichment levels.

Strategies to combat micronutrient deficiencies began in the 1940's in the United States and in some European countries, through the adoption of fortification strategies for a wide range of staple products (WHO, 2006). Iodine fortification of salt started between 1940-1950, and vitamin A fortification of sugar during 1970s. Both strategies have been successful in reducing the incidence of these deficiencies. "These programs have been carefully analyzed and their positive impact followed for more than forty years. Moreover, many of these strategies have been replicated with other micronutrients" (Dary, 2002).

According to the World Health Organization (WHO) & Food and Agriculture Organization (FAO) (2006), the process of fortification is defined as the addition of a nutrient to a food to improve its quality for people who consume it, generally for the purpose of reducing or controlling a specific nutrient deficiency. The prevalence of various vitamin and mineral deficiencies varies worldwide. The deficiencies tend to be dependent on age, gender and ethnicity. "Another reason for fortification is to prevent or reduce onset disease." (Kuczora & Richardson, 2012)

1.2.2.1 Methods of fortification

The method used to fortify foods should aim to improve stability of the micronutrients within the product, during both processing and storage. The appropriate method will depend on the micronutrient (or combination of micronutrients), product type and storage conditions. Some other important aspects include production, costs, and ease of access to equipment required for the selected method (Kuczora & Richardson, 2012). The methods listed in the following sections are used for fortification:

1.2.2.1a Premixes

A premix is commonly used when multiple micronutrients will be added to the product. Advantages include improving the homogeneity of added amounts of micronutrients to the product, and also the simplicity of inclusion of all micronutrients in the product (Kuczora & Richardson, 2012).

1.2.2.1b Direct addition

Direct addition is another method that is used is fortification (Kuczora & Richardson, 2012). This method is very easy and inexpensive. It is an alternative to preparing a premix and the more complex process of encapsulation. There are different factors need to be considered that such as particle size of micronutrient, the way the ingredients are mixed, and the time required for mixing. The process simply includes calculations, weight out and addition to the whole batch. Sometimes is difficult to control the exact quantity of the material being added. Processors should be careful of blend uniformity when using this method.

1.2.2.1c Encapsulation

Encapsulation is a special type of coating applied to small solid particles, liquid droplets or gases in order to provide protection (Sandoval, 2004). This type of coating acts as a barrier to avoid chemical reactions and/or to enable the controlled release of the ingredients. Encapsulation can be used for the extension of shelf life, fortification, controlled-release of nutrients, decreased hygroscopicity, the transformation of liquid flavors to powders, the stabilization during storage and transport under extreme temperature and humidity conditions improving organoleptic and functional properties of food products while limiting their interaction with other ingredients (Sandoval *et al* 2004).

The shell is specially designed to prevent diffusion of the core material into the food until a specific desired time. This shell can be made of one or more materials (Sandoval, 2004; Wilson, 2007). Materials used include polysaccharides such as gum arabic, modified starches, alginates and pectins; fats such as bees wax or proteins such as gelatins, whey proteins and sodium caseinates are used (Sandoval, 2004). Food ingredients can be micro-encapsulated through numerous methods but there is no single encapsulation process adaptable to all core materials or product applications (Wilson, 2007). Spray drying is the method most commonly used for vitamins, flavors, starter cultures, carotenoids, fats and oils and clouding agents. The steps include preparation of the dispersion or emulsion to be processed, homogenization of the dispersion, and atomization of the mass into the drying chamber (Wilson, 2007). Some other methods for encapsulation of vitamins and minerals include: extrusion, fluidized bed coating and coacervation (phase separation), (Wilson, 2007).

1.2.2.1d Other Technologies

Extrusion is the physical process in which raw materials, powders or grits, are pushed through a die to obtain products with a specific desired shape (Wang & Ryu, 2013). The extrusion of raw materials can occur with or without the presence of heat. Cold extrusion, involves low temperature, high moisture materials to create products such as pasta. Hot extrusion or cooking extrusion uses low moisture, high temperature, and small particle size ingredients to generate its final product. Hot extrusion is a continuous, highly energetic and efficient process that brings desirable reactions as well as nutritional improvements to the food matrix (Zhao et al., 2011). During the process, heat causes changes such as pre-gelatinization of starches, increased Maillard reaction, increased protein digestibility and changes in the amino acid profile (Souza & Andrade, 2002; Singh et al., 2007). Extrusion into pellets or snacks or the addition of nutrients matrixes into extruded products are also another way to add fortificants to food products. For example, an extruded product using corn, chickpeas, and bovine lung was created into a fortified snack to combat iron deficiency anemia among schoolchildren in rural Brazil using the cooking extrusion method (Moerira-Araujo, 2008). Asare and collaborators (2012) evaluated mixes of grains and legumes to elevate the protein content of extruded snacks.

1.2.3 Biofortification

Biofortification is the breeding and/or genetic engineering of plants to improve the value, variety, and bioavailability of nutrients they contain. Biofortification applies selective plant breeding using conventional techniques or modern biotechnology. Currently, the technique is being used to increase the presence and bioavailability of iron, pro-vitamin A, and zinc in major foods that people in developing countries primarily consume; these are known as staple foods (Daño, 2014; Hotz, 2007; Tarver, 2013).

Most regional staple crops seem to be adequate in caloric content but sometimes very poor in micronutrients, leading to micronutrient malnutrition, especially taking into consideration the monotony of certain regional diets. Biofortification projects that are underway include: iron-biofortified rice, beans, and sweet potato; zinc-biofortified wheat, rice, beans, sweet potato, and maize; provitamin-A fortified sweet potato, maize, cassava, and rice (Tarver, 2013). Globally, biofortification efforts are initiated and implemented by public institutions and funded mainly by public money. The most established international initiative in biofortification is HarvestPlus programme of the Consultative Group of International Agricultural Research (CGIAR) (Daño, 2014). This program is coordinated in conjunction by the International Centre for Tropical Agriculture (CIAT) and the International Food Policy Research Institute (IFPRI). HarvestPlus and its regional and national partners have evaluated and released nutrient-enriched food crops in more than 30 countries. They claim to have reached approximately seven million people worldwide and aim to reach more than 100 million people by 2018 (Daño, 2014). The first biofortified crop released by the program in 2007 was Vitamin A-enriched orange-colored sweet potato. For the most part, these projects have been received positively, but they are not without challenges that range from political to financial to agricultural to cultural. One of the main challenges of biofortified staple crops is farmer, grower, and consumer acceptance. For example, increasing the iron and zinc content of crops does not result in sensory changes, but boosting the content of provitamin A in crops results in noticeable changes in color and possibly taste (Hotz and McClafferty, 2007). Farmers may be reluctant to grow newly gold- or orange-hued crops that could be expensive to cultivate and difficult to sell, and consumers may be reluctant to purchase and eat food that looks unfamiliar and/ or tastes unusual (Meenakshi et al, 2007). Other types of hurdles include the approval of GMO releases (Daño, 2014). A number of developing countries in Asia and Latin America have adopted biofortification as a strategy to address undernourishment and micronutrient deficiency. The Brazilian Agricultural Research Center (EMBRAPA) works with research institutions across Latin America and the Caribbean in a regional biofortification and post-harvest processing programme called AgroSalud that targets farming families to grow biofortified crops, as well as consumers and beneficiaries (HarvestPlus, N.D.). AgroSalud started in 2007 and was meant to complement the HarvestPlus programme that began in Brazil three years earlier. It aims to increase the nutritional content of regionally important staple crops, namely iron and zinc in beans, maize, rice and sweet potato; tryptophan and lysine in maize; and β-carotene in orange-fleshed sweet potato and yellow maize (HarvestPlus, N.D.).

1.2.4 Food Relief Programs

According to Tarver (2013): "Food aid programs for developing regions tend to have a more targeted, customized approach to food fortification." Aid efforts before 1980 were focused on macronutrient-malnutrition as a result of a low calorie diet; for example: inadequate intake of protein, fats, and carbohydrates. Currently, many food aid organizations consider not only macronutrient malnutrition factors but also the dietary pattern and regional conditions that lead to monotony or affect acceptability by regional inhabitants (Tarver, 2013; WFP, nd.).

According to Tarver (2013), The World Food Programme distributes fortified blended foods. Fortified blended foods are partially precooked and milled cereals with added micronutrients; for example: *Super Cereal*® and *Super Cereal Plus*®; these are ready to use foods. Some of these tend to be peanut-based products fortified with vitamins and minerals (e.g., *Plumpy'Sup*™ and *Plumpy'Doz*™ by Nutriset); and micronutrient powders, which are tasteless powders containing the recommended daily intake of a range of essential vitamins and minerals that can be sprinkled on homemade food (e.g., *NurtureMate*™ by H.J. Heinz Co. and *Nutrifer*® by Sprinkles). These food products are formulated to address micronutrient deficiencies mainly in women and children.

Currently, the study of these fortified food products present some technical challenges, for example: 1) finding the nutrients that will be delivered in forms that the human body can absorb (highly bioavailable); 2) the fortified products have none of the unpleasant tastes or odors that can occur by adding fortificants to food and beverages; 3) the nutrient delivery systems are stable, preventing changes in a nutrient's chemical composition and 4) finding the most adequate type of packaging that will have a low permeability to water and oxygen and will be cost-friendly.

1.3 Sweet Potato Flour Production

Sweet potato is rich in nutrients, including carbohydrates, fiber, beta-carotene, vitamin C, phenolics and anthocyanins. It ranked highest in nutritional value among vegetables available in the United States. The amount of a specific nutrient in sweet potato varies among cultivars. It’s reported that the protein content can vary from 1.0% to 9.0%, and the fiber content can be as low as 0.7%, or as much as 3.4% on a fresh weight basis (Woolfe, 1992). Sweet potato flour (SPF) can be derived from this nutrient-rich crop. SPF is a nutrient-rich, shelf-stable product produced using simple processing methods that can be further improved with nutrient fortification

Flour production can be classified by the use of equipment involved (Odebode, 2004). One utilizes traditional and simple processing equipment: solar or mechanical driers such as cabinets, and the other one uses more expensive techniques: tunnels, drum, or spray dryers. These methods can further be classified as either cold or hot (Odebode, 2004). The Cold Processing Method involves peeling, washing and boiling of sweet potato roots. The boiled roots are put inside cold water and left to stand in cold water for 3 – 4 days. The water is then drained and washed to reduce the smell. The boiled roots are dried and then ground (Odebode, 2004).

The Hot Processing Method: After peeling, washing and boiling, the hot roots are soaked in hot water for 2 to 3 days. This helps to produce better and smoother flour. This flour can be prepared into paste and mixed for use in other preparations. The improved sweet potato processing technique involves the use of modern and expensive equipment. The stages include selection and weighing of tubers, washing, peeling, chopping and slicing, drying, primary grinding, attrition grinding and suction blowing and bagging (Odebode, 2004).

1.4 Scope of Analytical Techniques for Vitamin and Mineral Quantification

The following study proposes in Chapter 2 a method for creation of sweet potato flour, its characterization of thiamin, riboflavin, niacin, folic acid and iron and subsequent fortification to create a comparable micronutrient profile of enriched wheat flour. In order to quantify the amount of these micronutrients different analytical techniques were explored. It is possible to proceed with fortification by adding the necessary amounts once the quantification has been done. This section summarizes different possible methods for the analysis of thiamin, riboflavin, niacin, folic acid and iron and a final justification for the final selected method of analysis.

Procedures for thiamin extraction and quantification commonly used are: fluorimetry, gas chromatography (GC), high-performance liquid chromatography (HPLC) and microbiological assays (Ball, 1994; Eitenmiller, et al., 2008; McRoberts, 1954). All of the assays mentioned above follow an acid extraction to release the vitamin from their protein association and an enzymatic digestion to free thiamin from the phosphate esters in case of being found as TPP (Ball, 1994).

It is possible to use fluorimetry even when thiamin itself does not exhibit native fluorescence; thiochrome derivatives can appear in the presence of chemical oxidizing compounds (for example 0.1N HCl) at different pH (McRoberts, 1954). These thiochrome derivatives produce different fluorescent intensities that can be read at different wavelengths being the λ_{max} of peak around 432 and 435nm for the emission spectra (Ball, 1994). According to the same author, GC can be applied by determining the thiazole degradation product using the nitrogen-phosphorus detector or a flame photometric detector. HPLC can also be used; while being non-destructive, it can provide a greater chromatographic selectivity (Abdel-Kader, Z.,1992). It can be useful to determine both thiamin and riboflavin simultaneously (Ball, 1994).

Microbiological methods can be found for all of the B-group vitamins where previously test tubes with a specific type of *Lactobacillus* were inoculated (Zhang, 2014). These organisms require a supply of a specific vitamin for growth. The method is equally effective when utilizing microtiter plates instead of test tubes (Eitenmiller, et al 2008).

The microbiological methods, according to Abdel-Kader (1992) are very accurate; however, they have the disadvantage of requiring several days to obtain results. This method was selected because of the current laboratory conditions; where no HPLC was available.

For riboflavin quantification, direct fluorimetry can be used by measuring the relative fluorescence intensities of the three flavins: riboflavin itself, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). According to Ball (1994), HPLC applications are complicated as after the preparation with acid extraction, FMN and riboflavin are chromatographically separated and this vitamin determination would need to be done by converting all of the FMN to riboflavin and measuring the riboflavin peak or by calculating FMN and riboflavin separately and then report by summing both. The microbiological method, seem to be the most accurate way to quantify this vitamin. *Lactobacillus casei*, used in the microbial determination, according to Eitenmiller (2008), responds to the products of acid digestion: FMN, riboflavin and other isomeric riboflavin phosphates.

Niacin can be determined by colorimetry, HPLC and microbiological assays (Ball, 1994; Goldschmidt & Wolf, 2007). There are AOAC Official Methods for the microbiological determination and colorimetric techniques (Official Methods of Analysis, 2000). According to Goldschmidt & Wolf (2007), LC coupled with detection by mass spectrometry (MS) offers the potential for excellent sensitivity and specificity. MS however, requires an expensive and very specific equipment.

Folic Acid in the form of PGA and most naturally occurring folates can be detected by HPLC; preparation methods for this technique are described by Ball (1994). Other methods of quantification include the microbiological assay of total folate activity (Eitenmiller, et al., 2008). The radioassay and non-isotopic protein binding are also used; these methods are known as biospecific techniques (Zhang, 2014). All the above methods for quantification have often been described as complicated because of the multiplicity and diversity of natural folates. These methods are also complicated because of the instability of folates and reaction to enzymes and their existence in very low quantities in biological tissues (Ball, 1994). The versatility of HPLC seems to be ideal for food quality control, especially for the determination of vitamins (Zhang, 2014). However, microbiological assays have been recognized by the international official institutions for many years (Eitenmiller, 2008). The VitaFast® kits include a microtiter plate with specific microorganisms that are pre-coated in the plate wells, along with the medium and standard that have to be reconstituted with sterile water. These kits have been validated by the inter-laboratory studies using internationally available reference materials from NIST and AACC (Zhang, 2014).

Following the creation of this flour and the micronutrient analysis, Chapter 3 describes a prototype product developed, using sweet potato flour as the main ingredient in a noodle formulation. A 5 week shelf life study was conducted and among the variables analyzed were protein, lysine, moisture content and water activity, free fatty acids, β-carotene, texture: compressive extension and compressive load, color: L*, a*, b*. The main objectives of this project was to expand value-added uses of North Carolina's sweet-potato crop through the creation of a fortified sweet potato flour and the collection of scientific data on new product formulation that could potentially increase nutrition knowledge and consumption of sweet potatoes, as well as expand the utilization and market for sweet potatoes in a processed form.

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CHAPTER 2

PRODUCTION OF SWEET POTATO FLOUR AND ANALYSIS OF MICRONUTRIENTS

2.1 Abstract

Different adaptations, broad presence and high yield allow sweet potatoes to be a valuable crop that can be converted to valued-added products; among these is sweet potato flour (SPF). Wheat flour fortified with vitamins B_1, B_2, B_3, folic acid and iron can provide a significant amount of the Recommended Daily Allowance (RDA) of micronutrients to the population that consumes fortified wheat flour regularly in various food preparations. This study was designed to modify SPF by fortification to be equivalent to wheat flour in micronutrients. Vitamin content was determined in pre- and post-fortified SPF and compared with enriched wheat flour using VitaFast® Thiamin, Riboflavin, Niacin and Folic Acid assay kits. These kits determine the vitamin content microbiologically, utilizing microtiter plates coated with specific microorganisms that require a supply of each vitamin in their growth media. Data were analyzed with a 4-parameter equation from RIDA®SOFT or using the linear portion of the curve for microbial growth, which relates the light absorption by the bacterial suspension to vitamin content. The quantity of iron was analyzed by an acid digestion of the sweet potato flour followed by atomic absorption. Results showed between 0.51 mg and 0.66 mg/100 of thiamin; 0.48 and 0.848 mg/100 g of riboflavin; from 1.54 to 1.60 mg/100 g of niacin; 6.00 μg of folic acid and between 3.5 to 5 mg/100g of iron. Data provided 100% recovery of expected amounts for thiamin and riboflavin in sweet potato flour based upon the USDA Nutrient Database published values for baked sweet potatoes. The lower folic acid and iron values in SPF were expected based on previously published analysis in baked sweet potato. These results show that the amount of niacin (B_3) that should

be added to SPF to make a fortification level comparable to enriched wheat flour is 4 mg/100g; 0.15 mg/100 g additional fortification is needed for folic acid, and 0.5 mg/100g of iron. Sufficient recovery of thiamin and riboflavin show that there is no need to fortify with these vitamins. Further research is needed to ensure that these vitamins will be stable in SPF during processing, storage, and baking conditions.

2.2 Introduction

Sweet potato (*Ipomoea batatas* L.) is an important food crop and in many countries it is used as a staple food. It is cultivated in more than 100 countries. Domestication, artificial selection, natural hybridization and mutation, have resulted in many different varieties or cultivars. There is more diversity in sweet potato than in cassava and yam. These varieties differ in the color of the skin and flesh (white, cream, brown, yellow, red or purple), as well as in some non-visible cultivar aspects such as size of roots, depth of rooting, and resistance to disease (Woolfe, 1992). These different adaptations and broad distribution allow sweet potatoes to be an attractive crop that can either be consumed fresh or as an ingredient for processed food. Sweet potatoes are a versatile crop with growth characteristics that allow them to be considered a food security crop that is high yield in marginal conditions and can provide a significant amount of energy and protein with limited need for input of nutrients to soil. "Increasing recognition of the exiting potential which sweet potato holds for combating food shortages and malnutrition has resulted in intensified research efforts to enhance production and consumption" (Woolfe, 1992).

Sweet potatoes are ranked among the highest in nutritional value of vegetables available in the United States (Woolfe, 1992). They are known to be rich in nutrients that include carbohydrates, fiber, β-carotene, vitamin C, E and anthocyanins and contain moderate levels of iron, manganese and zinc (USDA, 2014). This crop, which is increasing in both acreage and yield, can be used to produce a value-added product such as sweet potato flour.

North Carolina has ranked as the No. 1 sweet potato producing state in the U.S., averaging at nearly 50% of the U.S. supply (North Carolina Sweet Potato Commission, 2014; USDA:Crop Production Annual Summary, 2014). The largest producing counties are: Sampson, Nash and Johnston, which are located in eastern N.C. (North Carolina Sweet Potato Commission, 2014). Factors accounting for the high yield are the hot, moist climate and rich, fertile soil. (North Carolina Sweet Potato Commission, 2014). Louisiana, Mississippi, Georgia and California also produce large sweet potato crops (USDA:Crop Production Annual Summary, 2014). Although sweet potatoes need only low input of nutrients, they grow better in temperate climates especially with full sunshine and regular moderate rainfalls. This crop prefers to grow in the 70-90 °F in the daytime and 55-65 °F at nights (Woolfe, 1992).

The growth in this industry comes from consumer interest in new foods, and to a large part, the successful marketing by the sweet potato processors and growers' organizations. Since 2007, there have been several new processing facilities constructed: a sweet potato processing plant based on microwave pasteurization (Yamco, Snow Camp, NC) and a production facility for sweet potato fries and wedges for restaurants (Conagra Foods Lamb Weston Division, Delhi, LA). These are indicative of the potential employment growth associated with this crop.

In contrast with these large processing plants, sweet potato flour can be made by cleaning, drying, and grinding sweet potatoes in either large or small-scale operations. Drying is an effective food preservation technique and there is a demand for sweet potato flour in in many bakery applications.

Methods for Sweet Potato Flour Production

According to Moncel (2014), flour is a type of powder resulting from the grinding of any kind of grain. Wheat is the one most commonly used; however, rice, potato, corn, and sweet potato can be turned into flours. Wheat flour is the most commercially available one (Alfaro, 2014). Sweet potato flour is the result of grinding the potatoes that have been washed, brushed, cut and dried. It is an orange powder that eventually turns white if left in room temperature, by a chemical reaction that implies either the loss or the transformation of the conjugated double bond of the beta-carotene structure found in sweet potatoes. Moreover, it is known that the sweet potato carotenoids exist in an all trans configuration. These have the highest pro-vitamin A activity among the carotenoids (Truong, 2010). While time affects the color but not the quantity it is also possible that some loss of carotenoids occurred. Carotenoids can possibly be isomerized by heat, acid, air or light during processing and storage. When carotenoids are exposed to heat, the molecule can be transformed from a cis configuration (typically at the 9, 13, and 15 carbon positions) (Truong, 2010). While isomerization does not affect the color, the baking process (which is a comparable process to the one carried out in the SPF creation) is reported to result in a 31.4% loss in the existing carotenoids (Chandler & Schwartz, 1988). In contrast to wheat flour, sweet potato flour has no gluten.

The sweet potato process in a traditional way involves the dehydration of sweet potatoes after the process of washing, peeling, slicing/shredding, blanching, soaking, pressing, and drying (Truong, 2010; Woolfe, 1992). The yield for this process after laboratory tests is around 20-25%. The losses occur during peeling and at slicing/ shredding but mostly during the drying process (Truong, 2010).

Flour production can also be classified by the use of equipment involved (Odebode, 2004). One method utilizes traditional and simple processing equipment: solar or mechanical driers such as cabinets, and the other one uses more expensive techniques: tunnels, drum, or spray dryers. These methods can further be classified as either cold or hot (Odebode, 2004). The Cold Processing Method involves peeling, washing and boiling of sweet potato roots. The boiled roots are put inside cold water and left to stand in cold water for 3 – 4 days. The water is then drained and washed to reduce the smell. The boiled roots are dried and then ground (Odebode, 2004).

During the Hot Processing Method, after peeling, washing and boiling, the hot roots are soaked in hot water for 2 to 3 days. This helps to produce better and smoother flour. This flour can be prepared into paste and mixed for use in other preparations. The improved sweet potato processing technique involves the use of modern and expensive equipment. The stages include selection and weighing of tubers, washing, peeling, chopping and slicing, drying, primary grinding, attrition grinding and suction blowing and bagging (Odebode, 2004).

Factors such as heating temperatures, processing times and storage (Kim, 1975) are also involved in the loss of vitamins in food products. An additional experiment in this chapter includes thiamin quantification on SPF. Thiamin, due to its native structure, is considered a strong base. It is ionized completely over the entire range of pH (NCBI, n.d.), where foods are normally encountered (Kim, 1975). In neutral or alkaline solutions, thiamin is destroyed rapidly because of decomposition of the thiazole portion. This principle allows thiamin determination in fluorimetry and HPLC methods (Ball, 1994, McRoberts, 1954). As the pH of the environment increases, thiamin becomes more susceptible to heat (Ball, 1994). In acid solution such as at pH 3.5, thiamin will withstand temperatures of 120°C for one-half hour without loss of activity (Kim, 1975). In neutral or alkaline solutions, thiamin is destroyed rapidly, because of decomposition of the thiazole portion. In dry form, the vitamin is very stable and is not sensitive to atmospheric oxidation.

The objectives of this study were to characterize a simple, scalable process for sweet potato flour fortification, and to retain nutrients and natural components to the greatest extent possible. Therefore, we focused on the use of a traditional method for flour production that used a dehydration process of chips in a mechanical drier instead of a boiling technique.

Micronutrient Determination

Micronutrient study patterns were determined using the standardized nutrient additives (iron, niacin, thiamin, riboflavin, folate) and level of fortification prescribed for enriched wheat flour, after adjusting for the content of these micronutrients found in sweet potato flour.

VitaFast® as a method for micronutrient quantification of Vitamins B_1, B_2, B_3 and Folic Acid

Vitamin content for thiamin (vitamin B_1), riboflavin (vitamin B_2), niacin (vitamin $B_{3)}$ and folic acid was determined in pre- and post-fortified SPF and compared with enriched wheat flour using VitaFast® Thiamin, VitaFast® Riboflavin, VitaFast® Niacin and VitaFast® Folic Acid assay kits. These kits determine the vitamin content microbiologically, utilizing microtiter plates coated with specific microorganisms (m.o.) that require a addition of a single vitamin in their growth media for the growth of the bacteria. The m.o coated in the plates belong to the lactic acid bacteria family *Lactobacillus*. *Lactobacillus* mutant strains have been developed that have an absolute growth requirement for one of the above mentioned vitamins. Therefore, this organism is suitable for the quantification of thiamin, riboflavin, niacin and folic acid in food and dietary supplements. As a group, the lactic acid bacteria are good candidates for vitamin analysis due to their genetic stability (resistance to undergo mutation), simple metabolic requirements, ease of maintenance and general safety as a non-pathogenic organism (Zhang, 2014).

Atomic Absorption for Iron Quantification

In atomic absorption spectroscopy, metal atoms are dispersed and vaporized into an air-acetylene flame. This process is called atomization and it is based on the principle of absorption of resonant radiation by an atomic vapor of an analyte sample (Rains, 1984; Karabegov, 2011).

The metal vapor absorbs radiation from the specific hollow cathode lamp in proportion to the number of atoms present (Skoog, 1996). Beer's Law is followed in the part-per-million range (mg of metal/liter of solution) for the quantitative determination of the concentration of an analyte element (Skoog, 1996). Flame atomic absorption is a fast and good analytical technique available for mineral samples, it offers high sensitivity and low cost for individual and high-volume analyses after the equipment investment (Rains, 1984; Karabegov, 2011). In this method, the main components are a primary light source, vaporizing system, monochromator, electronics and readout, and computer (Rains, 1984).

In brief, in atomic absorption spectroscopy, a liquid sample containing the metal analyte is aspirated into an air-acetylene flame, causing evaporation of the solvent and vaporization of the free metal atoms. Spectroscopic measurements are most easily performed with liquid samples; therefore, it is necessary to convert the insoluble solid matrix with the use of acid digestion. Proceeding atomization a hollow cathode lamp operating in the UV-visible spectral region is used to cause electronic excitation of the metal atoms in the cathode, and the absorbance of light generated by the lamp, which is proportional to the concentration of atoms of that element in the vapor phase, is measured with a conventional UV-visible dispersive spectrometer detector (Karabegov, 2011).

Costs of fortification

According to the Food Fortification Initiative (FFI, 2014), formerly the Flour Fortification Initiative, the cost of fortifying wheat flour is much lower than in the past. The total cost of adding the mandatory nutrients to flour in the USA (i.e. 6.4 mg/kg Vitamin B_1, 4.0 mg/kg Vitamin B_2, 52.9 mg/kg Niacin and 44.1 mg/kg Iron), is currently less than one US Dollar per metric ton of flour (FFI, 2014~~).~~

This is about 0.1 percent of the cost of flour in the stores, considering that enriched wheat flour costs in average around $1.99 in a packet containing 2 lbs (July, 2014). On the other hand, if the cost in the USA is calculated on a per person per year basis, and average wheat consumption is 205 g/person/day, the total cost of–fortification is US$ 0.07 per person per year (FFI, 2014).

This study evaluated the micronutrient levels that we needed in order to mimic the micronutrient flour profile of wheat flour required by legislation, with respect to vitamins B_1, B_2, B_3 and folate. Additionally, an experiment in this chapter included thiamin quantification on SPF with two different grinding sizes. During the coarseness/ fineness processing while grinding the SPF heat was involved and a thiamin determination was performed to determine any losses during the grinding process.

The micronutrient profile characterization of this product allows for an increase in nutrition knowledge and will potentially allow the diversification in consumption of sweet potatoes in a processed form. The bakery industry has a growing interest in substituting wheat flour with sweet potato flour (SPF), especially in the development of low glycemic index or gluten-free products. Flour fortified with micronutrients (vitamins B_1, B_2, B_3, folate and iron) can provide a significant amount of the Recommended Daily Allowance of micronutrients to the population that consumes fortified wheat flour regularly in various food preparations (Araujo, 2007).

2.3 Materials and Methods

Flour Production

For the purpose of this project the Covington variety was used to produce the SPF. 'Covington' is an orange-fleshed, smooth-skinned, rose-colored, table-stock sweet potato, developed by North Carolina State University (Yencho, G., et al, 2008). Selected sweet potatoes were stored in a refrigerated room maintained at 15 °C. The sweet potatoes were selected and cleaned in order to eliminate all the dirt. All damaged portions were removed to increase the quality in the process. Upon completion of the selection process the sweet potatoes were cleaned by washing them with tap water and brushing them gently. This was done carefully to avoid breaks on the skin of sweet potatoes.

Sweet potatoes were cut into thin slices with a semi-automatic slicer (Presto PS-10 Eau Claire, WI) with the blade at 3 mm (0.11 inches). There was no need to peel the potatoes s the skin of the sweet potato was extremely thin and contributes to the low glycemic index of sweet potato (Allen et al., 2012).

The slices or chips were dehydrated in an industrial cabinet dehydrator. This cabinet was manually constructed and dehydrated at a temperature of 60 °C for 12 hours. A built-in hammer mill for milling grain with a #14 screen was used to crush the chips into smaller pieces. The end product was similar to a powder with 4.72% moisture. For analysis, the flour-like product was further ground with the use of Foss™ Tecator™ Cyclotec™ model 1093 Sample Mill (Eden Prairie, MN) designed for uniform grinding with a mesh screen of 0.24 mm (0.009 inches). Sweet potato flour (SPF) was stored at -80° C to avoid enzymatic and chemical degradation, which could result, in a visible loss of color due to the transformation or loss of the conjugated double bond structure of beta-carotene.

For the additional quantification of thiamin grinder Rancilio HSD-ROC-SD (Italy) was used at settings: 40, 30, 20 and 10 mm. For particle size analysis, sieves of different measures were used in which flour was vertically passed and screened. Water Activity (aW) was measured using an Aqua Lab Model 4TE (Decagon Devices, Pullman, WA) and moisture content was determined using a moisture meter Computrac Max-50 (Arizona Instruments LLC, Chandler, AZ).

Nutrient Content Determination

Vitamin content of thiamin, riboflavin, niacin and folic acid was determined in pre- and post-fortified SPF and compared with enriched wheat flour using VitaFast® Thiamin, VitaFast® Riboflavin, VitaFast® Niacin and VitaFast® Folic Acid assay kits. Microtiter plates coated with specific microorganisms that require a supply of each vitamin in their growth media were supplied in each kit. The kits were purchased from Sigma-Aldrich (St. Louis, MO).

In brief, sample extraction was carried out with 1 g homogenized sweet potato flour in a 50 mL sterile centrifuge tube. For all vitamin extractions, 30 mL phosphate buffer freshly prepared (0.05 mol/L, 0.1% ascorbate, pH 7.2) were added. The samples were centrifuged using an IEC CL 31 multispeed, Thermo Scientific, (Waltham, MA). Tubes were filled to 40 mL with corresponding extraction solution (buffer), followed by extraction for 30 min in 95 °C water bath under gentle shaking. After that, the centrifuge tubes were chilled down quickly to below 30 °C in a mixture of ice and water, followed by centrifugation at 8000 x *g* for 10 minutes. Finally, the clear supernatant was further diluted in 1.5 mL sterile Eppendorf tubes with sterile water provided by the VitaFast kits to adjust sample concentration to match the standard curve. The microbiological test systems are used in accordance with the AOAC international norms, and are adapted from Official Methods 960.46 for Vitamin B_1, B_2 and B_3 and Official Methods 960.46 and 944.12, for Folic Acid (AOAC, 1960). A separate kit was used for each vitamin. The extraction followed the same procedure but differed in the dilution factors that were selected in accordance to the standards found in each kit. The diluted extracts and standards were distributed into the wells of a microtiter plate as follows: 150 μL of the respective medium was pipetted into wells followed by the addition of 150 μL of standards or diluted samples into the assigned wells. Then, the strips were covered with adhesive foil and incubated at 37 °C in the dark for 44–48 h in an incubator. The plates are coated with *Lactobacillus fermentum* (B_1), *Lactobacillus rhamnosus* (B_2 and folic acid) and *Lactobacillus plantarum* (B_3). The growth of the bacteria is dependent on the vitamin content of the sample, or standard. The analysis of the native vitamin content in solid and liquid samples requires an enzymatic extraction in order to release the analyte from the matrix. The following were the enzymes added to the SPF as described in table 2.1:

Table 2.1. Enzymes added for vitamin extraction

Essay	Enzymes	Quantity (mg/100mL)
Thiamin	Taka diastase Phosphatase of potato,	300 10
Riboflavin	Phosphatase of potato,	10
Niacin	Phosphatase of potato,	10
Folic Acid	Pancreatic enzyme	20

The incubation period at 37 °C was from 44 - 48 hour, as required for the microorganisms to reach the logarithmic growth. During this period the number of new bacteria appearing produced per unit time is proportional to the present population, which is correlated to growth rate. During the incubation period, the microtiter plate was covered with a film in order to prevent any debris or foreign material falling into the wells and contaminating the inoculum. Before the reading, the microtiter plate was sealed with adhesive foil, pressed down once more; then, placed upside down on a table to dissolve the microorganism thoroughly by shaking. After the plate was briefly shaken, the film was removed and absorbances of each well were measured using a microtiter plate reader (Thermo Scientific, Waltham, MA,) at 540 nm. The absorbance data reported in this study were analyzed RIDA_ SOFT Win data analysis program from R-Biopharm (Darmstadt, Germany).

Acid Digestion and Atomic Absorption

In preparation for atomic absorption spectrometry 0.5 g of SPF sample were rapidly digested with direct addition of 5.0 mL concentrated nitric acid with subsequent dilution with water to 10 mL volumetric flasks. This treatment was used in order to destroy the any organic matrix and solubilize the mineral.

In brief, the sample was prepared through microwave digestion using MARS 6 Microwave Reaction System (CEM Corporation, Matthews, NC). In the acid digestion, 0.5 g of the SPF samples and 5.0 mL of concentrated nitric acid were placed in the vessels and subjected to microwave heating in a closed Teflon™ vessel for the prescribed time and temperature. The samples were decomposed completely resulting in a clear solution in these conditions. Finally, the content was quantitatively transferred to 10-mL volumetric flasks and diluted with water.

An iron standard solution of 1000 μg $mL^{-1}$was purchased from Fischer Chemical Co. (Raleigh, NC, Product No, SI124). The actual iron contents were determined by flame-AAS Method 40-70 (Official Methods of Analysis, 1984). A Perkin Elmer, Atomic Absorption Spectrometer Model 3100 (Waltham, MA) was used for the analysis. The iron hollow cathode lamp was operated with a 12 mA current. The flame composition was acetylene and air; the burner height was approximately 13.5 mm, and the nebulizer flow rates were around 5.5–6.0 mL min^{-1}, according to manual.

Statistical analysis

Data for vitamin B_1, B_2, B_3 and folic acid were analyzed with a 4-parameter equation from RIDA_ SOFT Win or using the linear portion of the curve for microbial growth which relates the absorption given by growth to vitamin content. Data for iron analysis followed Beer's Law in the 0.5 to 10 part-per-million range (mg of metal/liter of solution). Acid extraction followed by atomic absorption was done for two different treatments in four samples. Therefore, the statistical differences were analyzed by a paired t-test; *P* values less than 0.05 were considered to indicate statistical differences.

2.4 Results and Discussion

Analysis for Vitamins B_1, B_2, B_3 and folic acid

For thiamin (B_1), as seen on table 2.2, results showed that quantity found was equal to 0.51 ± 0.37 mg/100 g. This amount was calculated while using the linear portion of the curve for microbial growth. The specified software found in the kit shows 0.66 mg/100 g when utilizing RIDA_ SOFT Win.

One limitation when working with these kits, is the sample preparation and extraction time variance, which increases test time, work load and reagent consumption. It is possible that if the extraction stage were held for longer time, more vitamin would have been extracted; therefore, a higher content in thiamin would have been reflected in the analysis. The analysis of the data was done using two different methods of calculation for a one time measurement with a triplicated sample: 1. The linear portion of the curve for microbial growth on the log phase and 2. RIDA_ SOFT Win, software; both calculation methods include the dilution factor in its final calculation and outputs the result in mg of thiamin per 100 grams of sample. Vitamin B_1 in fortified all-purpose wheat flour was also analyzed for this project. However, the readings were always much higher than the standards; therefore, the concentration couldn't be determined and the value presented in the fifth column is theorically taken from the CFR for flour enriched (FDA, Title 21, 2014). According to Zhang (2014), often the concentrations in fortified foods are higher than stipulated in the label because manufacturers tend to over-fortify the products in order to compensate for degradation during storage (Zhang, 2014).

Table 2.2. Summary of Findings: Vitamin and mineral analyzed, and type found natively in SPF along with the quantities found through the analysis of the microbial method; calculated manually using the linear portion of the curve or calculated through *RIDA_ SOFT Win.* Fifth column represents the quantity that should be added to enriched wheat flour according to Code of Federal Regulation (CFR). Column 6th and 7th are the amount and type to be added in order to create a fortified SPF comparable to enriched wheat flour

Vitamins and Mineral	**Type found in SPF**	**Quantity found via Microbial Assays utilizing linear portion of the curve /AA for Iron**	**Quantity found via Microbial Assays utilizing *RIDA_ SOFT Win* *1**	**Quantity according to CFR enriched wheat flour**	**Quantity to add in *100gr of SPF**	**Type of Vitamin for Fortification**
B1	Thiamine or TPP	0.51 ± 0.37 mg / 100g	0.66 mg / 100g	0.62 mg / 100g	0 mg/100g	Thiamine mononitrate
B2	Riboflavin	0.64 ± 0.10 mg / 100g	0.848 mg / 100 g	0.42 mg / 100 g	0 mg/100g	Riboflavin
B3	Nicotinic Acid or Nicotinamide	1.7 ± 0.13 mg / 100g	1.54 mg / 100g	5.5mg / 100g	4mg/100 g	Nicotinamide
Folate	Folic Acid	6.00 ± 0.04 mcg / 100 g	6.53 mcg / 100 g	0.18 mg / 100 g	0.18 mg/100g	Folic Acid
Iron	Ferrous Sulfate	3.6 mg ± 0.64 mg/ 100 g		4.4 mg / 100 g	0.8mg/100g	Ferrous Sulfate

Table 2.2. (Continued)

1 Corresponds to the only value given by *RIDA_ SOFT Win

The standard of identity for wheat flour (21CFR137.165) allows for reasonable overage with good manufacturing practices and does not specify an allowable maximum value. According to the USDA, National Nutrient Database for Standard Reference show 0.4 mg/100g for sweet potato, cooked, baked in skin, without salt (USDA: Basic Report: 11508, 2014), and 0.78 mg/100 g for Wheat flour, white, all-purpose, enriched, bleached. Figure 2 compares the values of data through analysis of two methods and information found in USDA database.

According to the mandatory legislation on fortification for wheat flour, when trying to reproduce and compare the sweet potato flour profile with wheat flour, additional thiamin may not be necessary due to the native amount in SPF (FFI, 2014).

The data for Riboflavin (B_2) showed 0.848 mg/100 g when data was analyzed with the linear portion of the curve, and 0.64 mg ± 0.10 / 100 g with the analysis by method 2, with RIDA_ SOFT Win (Table 2.2. column 4 &3, Row 2). The USDA database shows 0.42 mg/100 g for sweet potato, cooked, baked in skin, without salt and 0.494 mg/100 g for wheat flour, white, all-purpose, enriched, bleached (USDA: Basic Report: 11508, 2014 ; FDA, Title 21, 2014).

According to the mandatory legislation on fortification for wheat flour, 0.42 mg/100 g of riboflavin are needed in wheat flour (FDA, Title 21, 2014). When trying to reproduce and compare the sweet potato flour profile with wheat flour, additional riboflavin may not be necessary due to native amount in SPF-(FFI, 2014).

The data for niacin (B_3) showed 1.7 ± 0.13 mg / 100 g when data was analyzed with the linear portion of the curve, and 1.54 mg/100 g with the calculation by method 2, with RIDA_ SOFT Win (Table 2.2. column 3&4. Row 3). USDA database information found, shows 6.00 mg/100 g for sweet potato, cooked, baked in skin. According to the mandatory legislation on fortification for wheat flour, 4.0 mg/100 g of niacin are needed in wheat flour. When trying to reproduce and compare the sweet potato flour profile with wheat flour, niacin does not meet the wheat flour's standard of identity; therefore, fortification of around 3.5 mg/100g would be necessary (FFI, 2014).

The data for folic acid showed 6.00 μg/100 g when analysis was calculated with the linear portion of the curve, and 6.53 μg/100g with the analysis by method 2, with RIDA_ SOFT Win. For comparison, the USDA database shows 6.00 μg/100g for sweet potato, cooked, baked in skin, without salt (USDA: Basic Report: 11508, 2014). The low quantification for folate in this experiment could be due to the multiplicity and diversity of natural folates (Zhang, 2014; Ball, 1994) . These methods are also complicated because of the instability of folates and reaction to enzymes and their existence in very low quantities in biological tissues (Ball, 1994). In the U.S. and according to the U.S. Food and Drug Administration (FDA) the standards of identity for cereal grains labeled as "enriched" require mandatory addition of folic acid at 0.15 mg (FDA, 2014; Title 21, Code of Federal Regulations, 2001).

Acid Digestion and Atomic Absorption

Table 2.2 shows the concentration found for four different batches of flour using two different treatments of iron done in triplicates. For these, extraction through microwave digestion had different duration. There were two different cycles applied which in turn delivered two different colored solutions: Yellow treatment denotes a shorter cycle for acid extraction, this was found in the pre-set methods and denoted as express. Green treatment relates to a longer cycle, also selected from the pre-set method (45 minutes of digestion vs 1.5 hours). Analysis for iron was done using Beer's Law in the part-per-million range (mg of metal/liter of solution). The three different concentration measurements were done for each sample; therefore, for treatments with yellow color “a” concentrations were 5, 3.5 and 4 mg/100g and yellow “b” were 3 mg/100g for the 3 different readings. 3.5 mg/100g were recovered in Green “a” treatment and 3.5, 3 and 3.5 mg/100g were recovered in “b” Green.

Table 2.3. Iron concentration found for four different batches of flour put through acid extraction treatment with three replicates. Yellow and Green denote the colors resulting in two different digestion cycles selected from the Microwave Reaction System, MARS 6

Sample	Color Delivered According to Cycle	Concentration mg/100g		
1	Express: Yellow	5	3.5	4
2	Express: Yellow	3	3	3
3	Regular: Green	3.5	3.5	3.5
4	Regular: Green	3.5	3	3.5

The differences were analyzed by a paired t-test; p values less than 0.05 were considered to indicate statistical differences. The statistical analysis show *P* (T<=t= 0.63); therefore the two different sample preparation methods with shorter (express) and longer duration seem to be equal. The t-test found no significant difference between methods of analysis.

According to the mandatory legislation on fortification for wheat flour, 4.4 mg/100g of iron are needed in wheat flour. When trying to reproduce and compare the sweet potato flour profile with wheat flour, iron does not meet the wheat flour's standard of identity; therefore, fortification of 0.5 mg/100g would be needed (FFI, 2014).

An additional experiment to quantify thiamin was performed on a different SPF batch with two different grinding sizes. During the coarseness/ fineness processing heat was generated while grinding the particles and a thiamin determination was performed to determine any losses during the grinding process.

Table 2.4 Water Activity, Moisture content and pH on SPF

Variables	**Level 40 (large)**	**Level 30**	**Level 20**	**Level 10 (Fine)**
Aw	0.35 +/- 0.002	0.35 +/- 0.002	0.34 +/- 0.002	0.3 +/- 0.004
Moisture	5.37 +/- 0.15	5.21 +/- 0.09	5.72 +/- 0.11	5.42 +/- 0.25
pH	6 +/- 0.009	6 +/- 0.021	6 +/- 0.009	6 +/- 0.012

Results for sweet potato flour show pH at 6. Values according to Alban (2011), report pH of 5.58 ± 0.05. The acidity of the flour increases as time goes on storage, giving older flour high acid values (HrušKová, 2002) As seen on table 2.4, water activity (a_W) values for coarser particles are 0.35 ± 0.002 and 0.3 0.3 ± 0.0040, moisture content 5.37 ± 0.15 and 5.42 ± 0.25. a_W values reported by Alban (2011), show 0.387 ± 0.007 and moisture between 6.05 ± 0.16. Also the water activity depends on the size of the particle a larger particle size. Higher moisture contents, the acidity of the flour are increased as time goes on storage, old flour give high acid values.

Particle size was done through a sieve analysis to determine particle size distribution. Table 2.5 and 2.6 show the distribution for coarser and finer particles respectively. As seen in table 2.7, thiamin quantification was performed following these measurements.

Table 2.5 Results of sieve analysis for particle size on larger grinder setting (setting = 40 mm)

Particle Size Analysis				
Type	**40**	Initial quantity	67.3 g	
Mesh Size	Inches	Mm	Total	%
	0.223	5.66	2.3	3.49
	0.0937	2.36	42.7	64.73
	0.0707	2	4.8	7.28
	0.0469	1.18	9.5	14.40
	0.0394	1	1.2	1.82
	0.0197	0.5	3.3	5.00
	0.0165	0.425	0.84	1.27
	0.098	0.25	0.96	1.46
	0.021	0.053	0.37	0.56
	0.02	0.05	0	0.00
Total			65.97	

Table 2.6 Results of sieve analysis for particle size on smaller grinder setting (setting = 10 mm)

Particle Size Analysis				
Type	**10**	Initial quantity	71.6	
Mesh Size	Inches	Mm	Total	%
	0.223	5.66	0	0.00
	0.0937	2.36	0	0.00
	0.0707	2	0	0.00
	0.0469	1.18	6.6	10.00
	0.0394	1	4.6	6.97
	0.0197	0.5	14.3	21.68
	0.0165	0.425	6.2	9.40
	0.098	0.25	24.6	37.29
	0.021	0.053	12.5	18.95
	0.02	0.05	2.6	3.94
Total			71.4	

Table 2.7 Thiamine analysis. Plates D1-D3 belong to finer particle size and F1-F3 to coarser particle size.

plate	CODE	concentration	abs	dilution factor	weight
D1	10	0.166	0.112	10.000	1.0000
D2	10	0.164	0.107	10.000	1.0000
D3	10	0.168	0.117	10.000	1.0000
F1	40	0.190	0.173	10.000	1.0000
F2	40	0.166	0.113	10.000	1.0000
F3	40	0.169	0.121	10.000	1.0000

As seen on table 2.7, according to the curve of growth a dilution factor of 10 is suitable for reading, aiming the linear portion of the curve for microbial growth on the log phase which relates directly with the light absorption by the bacterial suspension to vitamin content. For these measurements, an average of 0.166 mg B_1/g and and 0.175 mg B_1/g were found in the samples analyzed. A student t-test (table 2.8), used to determine differences, was performed. According to the analysis there was no difference in thiamin content in the analyzed samples.

Table 2.8 T-test

Dilution	Concentration B1 (10)	Concentration B1 (40)
10	0.166	0.19
10	0.164	0.166
10	0.168	0.169
t-Test: Paired Two Sample for Means		
	Concentration B1 (10)	*Concentration B1 (40)*
Mean	0.166	0.175
Variance	4E-06	0.000171
Observations	3	3
Pearson Correlation	0.114707867	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-1.199112098	
P(T<=t) one-tail	0.176640909	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.353281819	
t Critical two-tail	4.30265273	

3.5 Conclusion

Enrichment and fortification are commonly used practices in the food industry. Nutrient quantities in enriched products follow a Standard of Identity stipulated in the Code of Federal Regulation. The standard of identity for wheat flour covers thiamin, riboflavin, niacin, folate, and iron addition. However, there is no Standard of Identity for SPF. This study determined that only niacin, folic acid and iron content in SPF are lower than the standard of identity for wheat flour. Fortification with thiamin and riboflavin would not be necessary for the nutrient content of SPF to equal or exceed that of enriched wheat flour.

The quantities of thiamin, riboflavin and niacin measured in SPF were in amounts expected based on USDA Nutrient Database published values for baked sweet potatoes. The results show that 4 mg/100 g and 0.5 mg/100 g are the amounts of B_3 and iron that should be added to SPF to make an enrichment level comparable to fortified wheat flour. The folic acid value was lower than expected in SPF, which could be due to degradation or an interaction reducing the microbial growth. Therefore, a complete addition of 0.15 mg/100 g of folic acid is required according to FDA standards of identity for cereal grains labeled as "enriched".

B_1-vitamin retention was measured in the sweet potato flour. The average concentration of thiamin in flour ground at the two particle size levels were 0.166±0.002 mg B_1/g and 0.175±0.013 mg B_1/g in small and larger particle flours. The vitamin content did not differ as a function of particle size of flour; indicating that the finer grinding did not cause heating to a level that would reduce nutrient retention.

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CHAPTER 3

APPLICATION OF SWEET POTATO FLOUR: DEVELOPMENT OF AN ALTERNATIVE FOR AFFORDABLE NUTRITION OPTIONS IN THE THIRD WORLD

3.1 Abstract

Disaster relief may require long-term feeding of nutrient-balanced food products. Recipients complain of lack of variety and little autonomy if ingredients are not those produced locally and familiar. The high levels of carbohydrates and β-carotene, in some varieties, and wide geographical distribution allow sweet potatoes to be used as a functional ingredient in dehydrated products. The purpose of this study was to develop a new malnutrition-preventing food product using ingredients commonly grown in tropical countries for use by relief agencies in disaster situations. A sweet potato, soy, and mung bean flour-based instant noodle was produced. After creating the product, chemical changes in an accelerated shelf life study are reported in this chapter. The noodles were stored up to 5 weeks at 28, 35, 45 and 55 °C. Statistical analysis showed decreases in protein ($P = 0.0027$), lysine ($P<0.001$), water activity (<0.0001), moisture content ($P<0.001$), compressive load (<0.0001), and β-carotene (<0.0001). The initial protein concentration of 20.7% decreased by 1.2% per week. Initial β-carotene concentration of 12 mg/kg decreased by only 0.4 mg/kg per week in noodles made with defatted soy flour and by 1.0 mg/kg per week in a higher fat formulation. Although these components were not stable over the testing period most of the change occurred between weeks 3 and 5. Fat content, compressive extension and measurement of L, A and B color seem to remain stable over time ($P>0.05$). The ingredients for these noodles are produced in many disaster-prone countries; therefore, the product can be easily manufactured and distributed within a 3-4 week shelf life. Packaging now used in some feeding programs should be modified to reduce water permeability.

3.2 Introduction

Nutrition Situation Assessment

According to the Food and Agricultural Organization (FAO, 2012), Asia and the Pacific region have the greatest number of food insecure people. It is reported that 578 million of the world's food insecure population live in this area, compared to 239 million in Sub-Saharan Africa and 53 million in Latin America and the Caribbean. This is comparable to the population of the United States, Canada, and the European Union combined, and account for nearly a seventh of the global population.

The earthquake in Southeast Asia in 2004 resulted in a tsunami disaster without any precedent, affecting mostly the countries of Indonesia, Thailand, Sri Lanka and India, the Maldives and the east coast of Africa: Somalia and Tanzania. In Indonesia, the province of Aceh, the closest to the epicenter, was the worst affected with more than 170,000 lives lost and millions of people who were affected (WFP, 2005). The World Food Program (WFP), part of the United Nations system, is an humanitarian agency fighting hunger worldwide that first responded to the region with approximately 403,000 kg of food including energy-biscuits (fortified with 9 vitamins and 5 minerals) and instant noodles. These were distributed to 200,000 survivors of the tsunami disaster as a measure to mitigate Moderate and Severe Acute Malnutrition (SAM) in an effort known as Treatment of SAM, with the objectives to save lives and protect livelihoods in emergencies (WFP, 2005). However, after several months of receiving these products, the recipients complained of a lack of variety in their diets because the same biscuits were distributed for several months and were foreign to their usual diets.

Different products such as nutributter® and plumpy'doz™ have been developed, especially for children. WFP has different classifications for them, especially with an application for susceptible populations such as children aged 6–59 months, pregnant and lactating women, and people in treatment for HIV and tuberculosis. Products can be targeted in one of the four main categories (WFP, n.d.):

1. Treatment of Severe Acute Malnutrition (SAM)
2. Treatment of Moderate Acute Malnutrition (MAM)
3. Prevention of Moderate Acute Malnutriton
4. Addressing Specific Micronutrient Deficiencies

However, in times of disaster, the WFP responds immediately with ready to eat foods that are recommended for nutrition interventions in emergency settings. The interventions seem to be well assimilated during the first days and can last for several weeks (Global Nutrition Cluster, 2011). However, there is a need for a progression with more variety of foods in the diets of the affected population once the basic services are restored. If services are not restored, it is very possible that recipients continue to be fed with these products for extended periods of time.

After a first intervention utilizing high-energy biscuits or instant noodles the progression is possible with an option such as packaged meals. An option for these type of meals is a mixture where the main ingredients are rice, soy flour, salt, dried vegetables, and a sachet of powdered vitamins and minerals. The meals are intended mainly for malnourished school-aged children in low-income countries; however, in a food insecure setting, it may be necessary to feed a complete family. This type of meal is packaged and paid for by volunteers in the U.S., so they must be low cost and made of ingredients that can be packaged by several people in an assembly line. The meals are shipped from the United States to different countries. Currently, they are known to have a two year long shelf life (Renquist, 2013). Other types of improved food products that are made of several dry ingredients (made of several components) similar to the packaged meal concept are improved porridges made from micronutrient-fortified corn-soya blend, sugar and water. Lunch packages can be made of maize meal and beans with vegetable oil and salt, sometimes substituted with rice and sometimes including fruits and vegetables from school gardens. Implementation of school lunches increased cognitive development along with increased school attendance, since meals served at school in an incentive to attend regularly (Adelman, 2008). One-pot meals are meals that require cooking and may contain grain(s), legume(s), oil, milk, and added micronutrients (Avula et al., 2011; de Pee & Bloem 2009).

Pre-cooked or extruded porridges with added micronutrients, are also available. These usually require the addition of hot water for preparation (Lopez de Romana, 2000; Lutter et al., 2008).Some other types are snacks that contain a few components in packets for individuals, such as biscuits and a drink (van Stuijvenberg et al. 1999). Other prepared foods made of local ingredients, such as, githeri (maize, beans, greens) sometimes with meat added or something similar to a cheese sandwich (Bentley, 1991).

Noodles are widely consumed throughout the world and their global consumption is second only to bread (Jayasena, 2010). In this study a mixture of sweet potato flour (SPF), xanthan gum and mung bean flour was used to formulate a new product with the purpose of developing a new malnutrition preventing food product using ingredients commonly grown in some tropical countries, especially for the South East Asia region, for use by relief agencies to increase the overall intake of nutrients in disaster situations.

3.3 Materials and Methods

Fifty grams of Covington SPF previously prepared in our pilot plant, were mixed with 37.5 g of soy flour (SF) (Bob's Red Mill Natural Foods, Milwaukie, OR). For the purpose of this study two kinds of SF were used: low fat SF (7.8% of fat content) (LFSF) and regular SF (21.4% of fat content) (RSF). SPF with LFSF and SPF with RSF, were mixed with 12.5 g of mung bean flour (Bob's Red Mill Natural Foods, Milwaukie, OR) and with 2.0 g of xanthan gum (Bob's Red Mill Natural Foods, Milwaukie, OR). The gum was used as a pseudoplastic ingredient that allowed the noodles to stick together, in the absence of wheat gluten. Water (100 mL) was added slowly, as water amount is dependent on absorption by flour. This mixture was combined in SIMAC MX700 PastaMatic (Philadelphia, PA) and extruded with a Kitchen Aid (St. Joseph, MI) pasta maker with a die of spaghetti size included in the pasta maker. During extrusion, noodle strands were obtained manually by putting hand pressure in front of the noodle strands coming out of the die. The noodle strands were cooled to room temperature, cut into approximately 20-cm lengths and dried in a Thermo Scientific ELED PR305225M Precision Mechanical Oven (Waltham, MA,) at 70°C for 7.5 hours. After drying, the noodles were packed in a co-extruded polyethylene (PE) and polyethylene terephthalate (PET) bags (40 g per package) and heat sealed with an ULINE 8" Heat Sealing Impulse Manual Sealer Machine PFS-200 (Pleasant Prairie, WI). The total yield resulted in 52 packed samples that were used in a five-week shelf-life study.

Study Design

An accelerated shelf life study that related temperature with a time projection was designed. For this, noodles were stored at room temperature (approximately 18°C), at 35°C, 45°C and 55°C. Each week an array of nutritional content variables were measured.

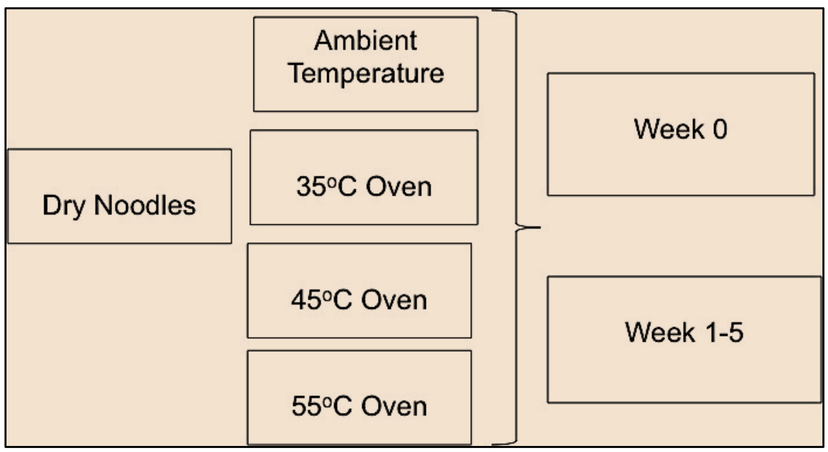

Figure 3.1. Accelerated Shelf Life Study Design

The variables and its modification for the purpose of this study could translate to nutritional content degradation or quality alteration over time. The variables analyzed were protein, lysine, moisture content and water activity, fat, β-carotene, texture: compressive extension, compressive load, and color with the variables L*, A*, B*.

Sample Preparation

Forty grams of the prototype noodle product were collected at each time and temperature and were immediately ground with the use of Cyclotec Sample Mill Grinder model 1093 (FOSS sample mills, Eden Prairie, MN); which is designed for fast and uniform grinding through a 60 mesh screen (0.24 mm).

Analytical methods

I. Protein, was measured using a bicinchoninic acid (BCA) Protein Assay Kit (Pierce™ Chemical Company, Rockford, IL).

II. The moisture content was determined using a moisture meter Computrac Max-50 (Arizona Instruments LLC, Chandler, AZ).

III. Free Fatty Acids (FFA) method was carried out using the using the American Oil Chemists' Society (AOCS) official methods AC 5-41 (AOAC, 2000). .

IV. Extraction of β-carotene was based on a method described by Chandler and Schwartz, (1988). In brief, β-carotene was extracted with a mixture of hexane and acetone solvents (organic extraction) and spectrophotometrically quantified, as follow:

5 grams of SPF were mixed with 25 mL of methanol for 1 minute. The mixture was filtered under vacuum through a funnel. 50 mL of a hexane–acetone (1:1) mixture was prepared, added and stirred, filtered again with 3 repeated additions of hexane–acetone (1:1) mixture until the filter cake turned white.

The colorless residue in the funnel was washed two more times with 25 mL of methanol and then by 50 mL of the hexane–acetone mixture. All of the extracts were combined in a 250 mL separatory funnel and washed with water. 5 mL of saturated sodium chloride solution were added to the funnel to facilitate phase separation. The aqueous phase was discarded and the upper layer was transferred to a 50-mL volumetric flask and made to volume with hexane. Samples were analyzed using a spectrophotometer at 450 nm and readings were recorded. Quantities were determined as follow: β-carotene (μg/mL) = A / (e * 10^{-4}) * DF, where A = absorbance, e = 2592 is the molar absorptivity of β-carotene, and DF is the dilution factor.

V. Texture

The texture of the noodles was measured with an Instron 5542 (Norwood, MA) testing machine according to a 3 point bend (far/rate and suspension distance) compression method of 25% at a speed of 1 mm was applied per sec. Both the maximum load and the maximum compressive stress were determined.

VI. Water Activity (aW) was measured using an Aqua Lab Model 4TE (Decagon Devices, Pullman, WA). Each sample contained 3.5 g, the cap was used to prevent any additional moisture into the sample that could alter the measurement.

VII. Lysine determination was performed using the method of Iwe, (2004) with modifications. In brief, 0.5 g of finely ground sample were measured into a 15 mL plastic culture tube. To this 7.5 mL of water were added. The supernatant was vortexed (20 min) and centrifuged to clarify. 0.5 mL of the solution were taken and added into another tube and we added 1.5 mL of 16%

sodium dodecyl sulfate (SDS) dissolved in water. This solution was allowed to stand overnight. A stock of L-lysine in water at 50 µg/mL was prepared and a standard curve over the range of 1 to 10 µg/mL was prepared by dilutions in water.

Into a black 96 well microplate, 250 µL of o-phthaldialdehyde (OPA) solution purchased from Sigma-Aldrich (St. Louis, MO) were added followed by 10 µL sample. Samples were read after 2 min of shaking at 25°C. The reading was done with Tecan Safire 2 (Tecan Trading AG, San Jose, CA) at an excitation wavelength 340 nm and emission wavelenth 455 nm. For the calculations, averages of all the blank readings and the average value from each standard and sample reading were subtracted. A dilution factor of 3380 and the following equation was used to calculate the results:

Available Lysine (µg/g sample) = concentration from the curve (µg/mL) x 3380/sample weight (g)

VIII. Color variables were measured with Hunter Lab D25 L (Hunter Associates Laboratory, Inc, Reston, Virginia) optical colorimeter equipment, using the colorimetric tristimulus scale (L * a * b *), wherein the L* axis measures the clarity of 0-100 (black = 0 and white = 100), the a* axis (negative = green and positive = red) and b* axis (negative = positive = blue and yellow). For each samples three color readings were determined and were averaged.

IX. Two different nutritional labels were created with the use of Genesis software for R&D Product Development and Labeling from ESHA (Elizabeth Stewart Hands and Associates)

Research. Software was facilitated and available by research extension specialists in Entrepreneur Initiative for Food (ei4f) at the FBNS Extension Program.

3.4 Results and Discussion

Table 3.1 shows a summary of statistical significance in columns for the variables analyzed during a 5 week period with data collected during week zero (first days), week 1, 2, 3 and week 5 (complete data presented in Appendix A). The significance probability for main effects and interaction terms are shown in rows. Empty cells indicate no significant effect (*P* > 0.05). The 3-way interaction was also tested in the model, but was non-significant for all variables. Assuming that our null hypothesis is no effect of: week (time), temperature, ingredient or the interactions between week and ingredient or week and temperature on each variable measured (protein, lysine, moisture content and water activity, fat, β-carotene, texture: compressive extension and compressive load, color with the variables L, A, B); with a *P* value of more than 0.05 we would accept the null hypothesis and *P* value equal or less than 0.05 we would reject the null hypothesis. Table 1 shows the *P*-value for the variables that exhibited a significant difference.

Table 3.1. *P*-Value for different variables analyzed

	Macronutrients						Texture			Color		
	Moisture (%)	Water activity	Protein (%)	Fat (%)	Lysine (mg/g)	Carotene (mg/100g)	Delta Energy	Compressive extension (N)	Compressive load (N)	L	A	B
Week	<.0001	<.0001	0.0027		<.0001	<.0001			<.0001			
Temperature	<.0001	0.0259				<.0001	<.0001		<.0001	0.0004		
Ingredient	0.0234	<.0001			0.0215	<.0001	<.0001		<.0001	<.0001		<.0001
Week*ingredient		0.0353				0.0215						
Week*temperature		<.0001										

The analysis for protein, in Figure 1, shows that week was significant (*P*= 0.027). There was a constant increase from week zero to week three and an abrupt decrease until measured again on week five. This increase is possibly related to a loss in moisture that interfered with the protein assay. Noodles became harder during the experiment and the apparently lower concentration in protein could have been due to a lower solubility of the proteins in the assay buffer. Protein can remain stable over the time; however, it is often difficult to extract. Difficulty in solubilizing the proteins would result in lower measured values of available protein.

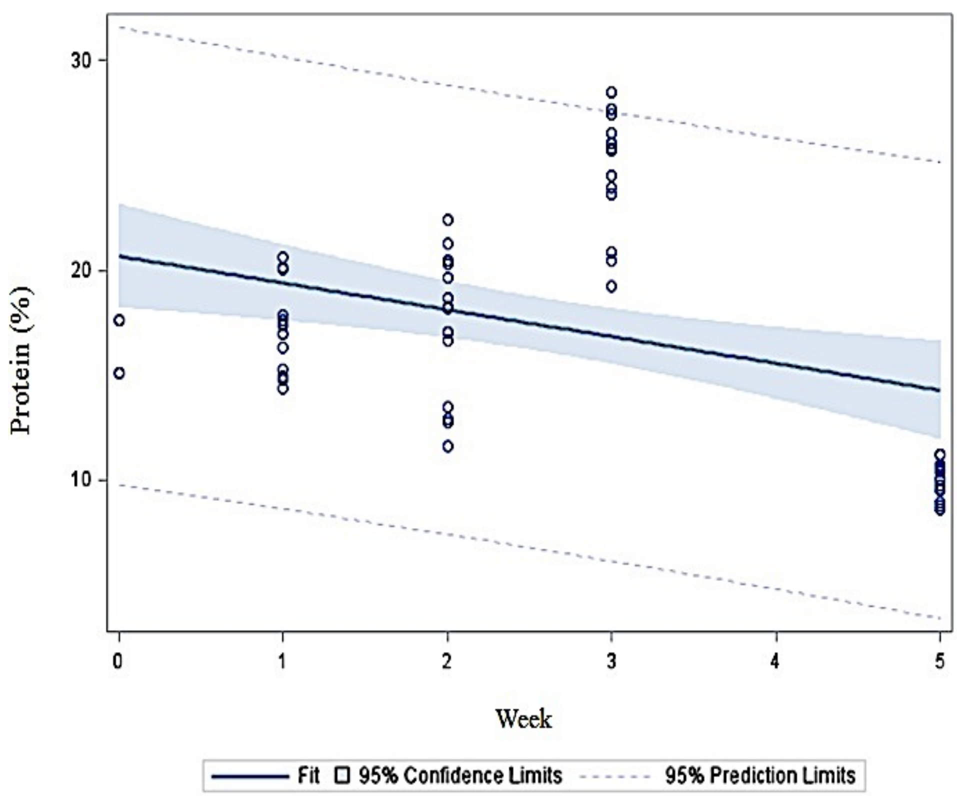

Figure 3.2. Protein Analysis

Measurement of lysine predicts loss of nutritional value due to Maillard browning (Martins, 2001 ; FAO, Agriculture and Consumer Department, nd.). Lysine residues are composed of an aliphatic chain with an amino group (a primary amine) attached to the functional group. This amino group is highly reactive and often participates in reactions that involve enzymes or non-enzymatic chemical reactions such as the Maillard reaction. The Maillard reaction involves bond formation between the carbonyl group of a reducing sugar and the nucleophilic amino group (Martins, 2001). As seen in Table 3.1, week (*P*< 0.001) and ingredient (*P*= 0.0215) were significant. The measurement for lysine during the first week was lower than the consequent measurements decreasing over time from week 0 to 5.

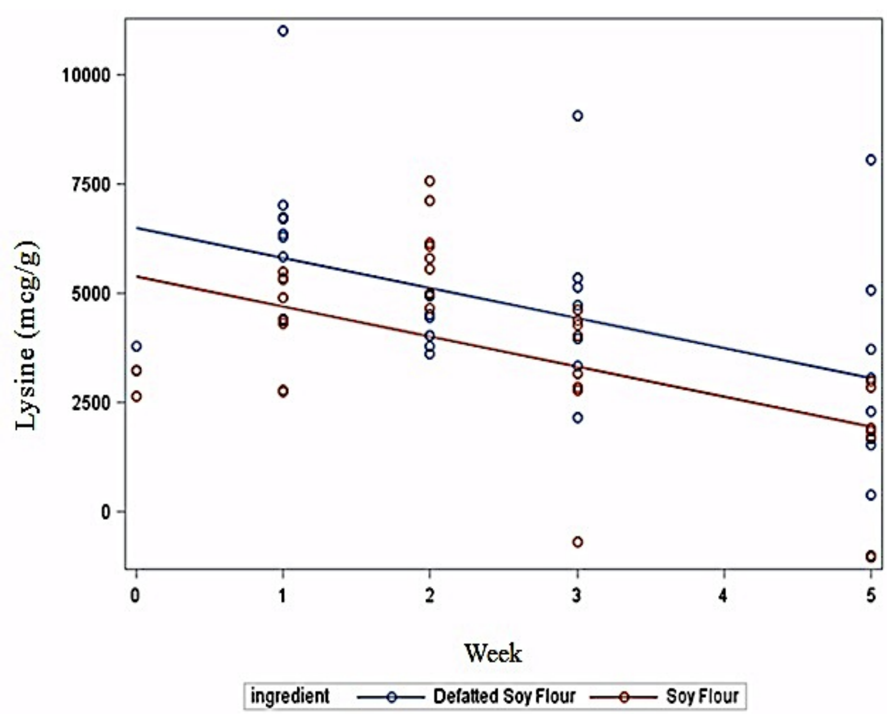

Figure 3.3. Lysine Measurement Analysis over Time

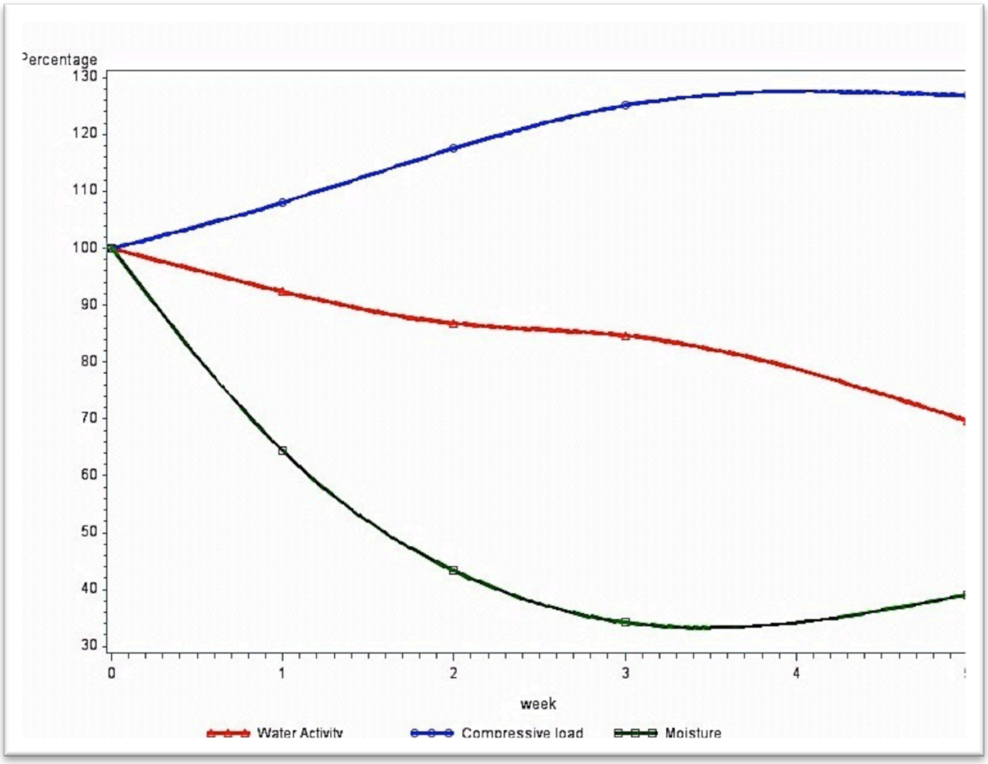

Figure 3.4. Water Activity, Compressive Load and Moisture Analysis over Time

Figure 3.4 shows the effect of week for moisture and water activity. Significant variables include: week (P<0.001), temperature ((P <0.001) and ingredient ((P= 0.0234). The formulated noodles lost moisture as they dried in the shelf life study. With respect to water activity, all terms: week (P<0.001), temperature (P=0.0259), ingredient (P<0.001) and including interactions: week and ingredient (P= 0.0353) and the interaction between week and temperature (P<0.001) were significant. Compressive load is a measure of the force applied to the noodles before fracture. For this variable, all terms: week, temperature and ingredient (P<0.001) were significant. Noodles become harder to fracture. As moisture and water activity decreased, the compressive load, or hardness of the noodles, increased.

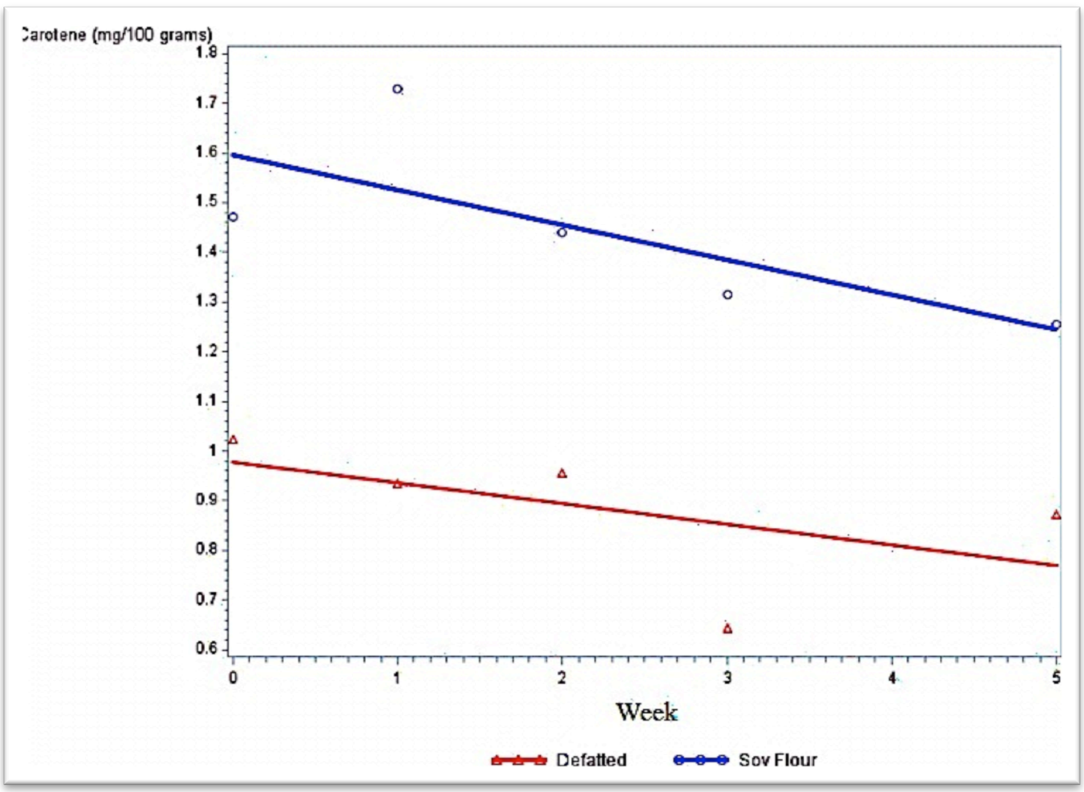

Figure 3.5. β-Carotene Analysis

Figure 3.5 shows that for *β*-carotene analysis, all terms were significant: week, temperature and ingredient (P<0.001) including week x ingredient (P= 0.0215). The significant week x ingredient interaction is evident because the slopes differ between the two ingredient formulations. According to results, *β*-Carotene tended to decrease over the studied variables.

An initial content of 1-1.6 mg/100g is significantly lower than the values found in the literature. The range of β-carotene content in cooked orange-fleshed sweet potatoes is reported to be 6.7 – 16.0 mg/100g fwb (Truong, 2010). β-carotene content is associated with variety of sweet potatoes. An important characteristic in varieties is reported by Javeria (2013), regarding genes and genes expression. Some varieties of sweet potatoes contain promoter genes for β-carotene in larger quantities, so those varieties have more β-carotene production (Ben-Dore, 2005; Javeria, 2013). The crop type and agronomic conditions also have an effect in β-carotene production favored by the light intensity received. Therefore, roots may vary in carotenoid content depending on the area where the sweet potatoes were grown. Being a warm season crop, getting maximum light intensity produces large amounts of β-carotene. Manure and fertilizers also played an important role in quantification. Javeria (2013) reports that fertile soils may favor the production of β-carotene. Topography is also a relevant factor for quantification, biosynthesis of β-carotene in fruits and vegetables are favorable in high altitude lands.

Variation in our results could be due to the week-to-week changes in the chemicals and solutions, lab conditions, and methodology while carrying out the extraction. In this sense detailed extraction while washing and the time in carrying out the procedure could have played an important role in obtaining such lower values over time.

Instrumental Color

Both L* and b* values, representing from light to opaque and yellow to blue components, respectively, varied, while the a* value that represents the red to green component was not altered by the weeks, temperature or ingredient. SPF contains naturally occurring yellow pigments that imparted yellowness to the product when mixed with the other ingredients (soy flour and mung bean flour) used in the formulation of the product. L* value represents lightness and the finished product was darker than control. This might be due to Maillard reaction, which was also demonstrated with the decrease of available lysine. Yellowness (denoted by b* values) remained constant; however there was a statistically significant decrease which tend to show noodles with a more green or less red color.

NUTRITION LABEL

For the prototype product, a serving portion of 100 grams equal to one cup has been estimated. Figure 3.6 and 3.7 correspond to the nutrition labels with defatted and regular soy flour as the only differential ingredient. These labels were generated from the ingredients, with the use of Genesis software for R&D Product Development and Labeling.

Nutrition Facts

Serving Size 100 g

Amount Per Serving

Calories 360	Calories from Fat 30
	% Daily Value*
Total Fat 3.5g	**5%**
Saturated Fat 0.5g	**3%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 5mg	**0%**
Total Carbohydrate 65g	**22%**
Dietary Fiber 8g	**32%**
Sugars 5g	
Protein 20g	**40%**
Vitamin A 440% •	Vitamin C 35%
Calcium 15% •	Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g
Protein		50g	65g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 3.6. Nutritional Label for Prototype Product Containing Defatted Soy Flour

Nutrition Facts

Serving Size 100 g

Amount Per Serving

Calories 380	Calories from Fat 70
	% Daily Value*
Total Fat 8g	**12%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 10mg	**0%**
Total Carbohydrate 66g	**22%**
Dietary Fiber 5g	**20%**
Sugars 0g	
Protein 16g	**32%**
Vitamin A 440% •	Vitamin C 35%
Calcium 10% •	Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g
Protein		50g	65g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 3.7. Nutritional Label for Prototype Product Containing Regular Soy Flour

According to the nutritional labeling software, products with a serving size of a cup as a cooked portion seem to provide a similar amount of calories (360 vs 380 Kcal). The amount of total fat in grams increases from 3.5 to 8 when changing from defatted soy flour to regular soy flour. Protein content is higher in the product containing defatted soy flour, 16 grams versus 20 grams in 100 grams of product. According to Global Nutrition Cluster (2011) World Food Program Specification for MAM, this type of meal generally is expected to provide 500 kCal and 13 g protein per serving. The developed product exceeds the amount of protein required and caloric content seems to be adequate while considering a cup of noodles is a regular portion for an adult to consume in one meal.

3.5 Summary

The combination of SPF, soy flour and mung bean in combination with xanthan gum as a pseudoplastic ingredient allowed the production of noodles that can be easily manufactured and distributed in a very few weeks. This product can provide nutrition and variety to diets of recipients in some disaster areas, for example areas of South East Asia prone to tsunami events.

Throughout this five week shelf life study that analyzed several different variables, it was apparent that the combination of multilayer packaging materials used (co-extruded polyethylene and polyethylene terephthalate bags) allowed moisture and gas permeability. This resulted in a decrease of most of the variables (nutrients) measured for this prototype product. Our suggestion to relief agencies is to carefully select a type of packaging that would reduce water permeability; then, this product could better result in a protein fortified nutrition option. Ethylene vinyl alcohol (EVOH) is a copolymer of ethylene and vinyl alcohol and might be the best choice. This plastic resin is commonly used as an oxygen barrier in food packaging; it is a better option than other plastics at keeping air out and allowing flavors to remain longer, it is transparent and water, oil, and solvent-resistant, flexible, moldable, recyclable, and even printable. Its drawback is that it is difficult to make; therefore, it is more expensive than other food packaging.

Although this food product seems to be an affordable and nutritious option, there are not enough micronutrients in the major product ingredients, including those that predominantly come from animal source foods, such as B_{12} and zinc. However, the product created could allow relief agencies to add nutrition and variety to diets in disaster areas, if more research is done to add the needed panel of micronutrients in a fortified product. Further investigation is required prior to implementation and consumption.

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APPENDICES

Appendix A. SAS Analysis of shelf life study of sweet potato noodle product

The SAS System

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
1	0	28	Defatted Soy Flour	17.613762486	3220.4418165	0.0960490463	4.385
2	0	28	Defatted Soy Flour	17.613762486	3790.6108464	0.2785950632	4.4425
3	0	28	Soy Flour	15.083240844	2637.8075754	0.1838714557	2.8375
4	0	28	Soy Flour	15.083240844	3236.3920767	0.1652507188	2.6325
5	1	28	Defatted Soy Flour	14.881889764	4381.6272505	0.1144480519	2.57
6	1	28	Defatted Soy Flour	20.044994376	6743.4468653	0.1007142857	4.45
7	1	35	Defatted Soy Flour	14.746906637	6710.2373844	0.0946308725	2.65
8	1	35	Defatted Soy Flour	17.817772778	6295.5339001	0.0719387755	3.1
9	1	45	Defatted Soy Flour	20.112485939	11010.017653	0.1040590406	2.32
10	1	45	Defatted Soy Flour	14.375703037	7014.8625705	0.1007142857	2.94

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
1	0.46355	0.00096625	2.80312075	1.6666615	0.9465755534
2	0.462	0.000919	3.52646925	1.811599	1.1028259073
3	0.3974	0.0003585	2.28107425	1.45769875	1.3944761243
4	0.404125	0.00038025	2.95630525	1.560341	1.5494551635
5	0.4387	0.000675	5.538531	1.903421	0.3925509233
6	0.4402	0.000736	5.784796	1.901315	0.5886589656
7	0.4184	0.000587	5.597265	1.917086	0.680492916
8	0.431	0.000715	5.813	1.784849	0.8032030269
9	0.453	0.000535	6.172953	1.817274	0.9716288311
10	0.4143	0.000543	5.624422	1.780264	1.2850339818

Obs	L	a	b
1	36.2525	9.5825	22.845
2	38.150833333	9.5775	23.805833333
3	47.2475	9.6591666667	33.118333333
4	46.408333333	10.0875	33.314166667
5	40.743333333	10.303333333	26.356666667
6	38.216666667	9.9633333333	25.23
7	41.666666667	10.406666667	27.633333333
8	37.56	9.4733333333	24.623333333
9	37.576666667	10.403333333	24.43
10	37.85	9.82	24.776666667

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
11	1	55	Defatted Soy Flour	17.345331834	5840.0830223	0.1254448399	1.72
12	1	55	Defatted Soy Flour	15.253093363	6371.4873791	0.1044444444	1.73
13	1	28	Soy Flour	20.652418448	5312.2239167	0.1674946004	2.53
14	1	28	Soy Flour	16.33295838	4325.2385682	0.0855582524	2.7
15	1	35	Soy Flour	14.949381327	4892.7201258	0.1104026846	2.78
16	1	35	Soy Flour	17.480314961	5360.9191326	0.0988317757	2.42
17	1	45	Soy Flour	16.940382452	5481.7979403	0.1045550847	1.73
18	1	45	Soy Flour	17.581552306	4416.4470884	0.1089403974	2.03
19	1	55	Soy Flour	16.940382452	2785.7460271	0.106587473	0.6
20	1	55	Soy Flour	17.581552306	2765.8805555	0.1158450704	0.55

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
11	0.3525	0.000403	5.214421	1.933658	1.3665868159
12	0.3567	0.000402	5.550296	2.00032	1.4019273082
13	0.3974	0.000365	4.941704	1.756297	1.4250531517
14	0.4123	0.000398	5.155921	1.541728	1.5508535125
15	0.3997	0.000385	5.551891	1.496717	1.5590778671
16	0.3932	0.00032	5.236891	1.515474	1.8134310274
17	0.3728	0.000289	5.369735	1.465811	1.7611248905
18	0.3937	0.000303	5.084781	1.518975	1.8941386721
19	0.3567	0.000344	5.364344	1.846569	1.8678426658
20	0.3536	0.000357	5.150454	1.911347	1.957231962

Obs	L	a	b
11	38.766666667	10.783333333	26.263333333
12	37.61	10.263333333	24.686666667
13	47.653333333	10.02	35.34
14	47.533333333	10.026666667	33.66
15	47.48	9.2966666667	34.01
16	47.57	10.423333333	34.42
17	47.62	10.27	34.133333333
18	48.18	10.066666667	33.943333333
19	47.133333333	9.81	33.77
20	45.756666667	11.036666667	32.656666667

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
21	2	28	Defatted Soy Flour	16.670416198	4976.9712355	0.1855263158	3.08
22	2	28	Defatted Soy Flour	11.642294713	4928.9139771	0.3204545455	3.13
23	2	35	Defatted Soy Flour	20.483689539	4516.3685166	0.47	2.17
24	2	35	Defatted Soy Flour	18.22272216	4043.8928864	0.1258928571	2.32
25	2	45	Defatted Soy Flour	18.661417323	4445.5767799	0.267721519	0.63
26	2	45	Defatted Soy Flour	12.755905512	4051.0004136	0.231147541	0.72
27	2	55	Defatted Soy Flour	22.373453318	3605.1779283	0.188	0.49
28	2	55	Defatted Soy Flour	17.041619798	3805.7273936	0.188	0.38
29	2	28	Soy Flour	13.498312711	5821.0222621	0.3027607362	2.16
30	2	28	Soy Flour	17.075365579	7115.5573802	0.2419117647	2.52

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
21	0.464	0.000858	4.546048	1.84066	0.8407507983
22	0.4452	0.000693	4.212485	1.997852	1.0360940964
23	0.4089	0.000523	4.199453	1.968513	0.7948743219
24	0.3907	0.000384	4.088376	1.868809	0.9614889481
25	0.3634	0.000302	4.004282	1.815288	0.9419743783
26	0.3693	0.000268	3.860312	1.820393	1.0039356705
27	0.3255	0.000496	4.256781	2.248598	0.9792628512
28	0.324	0.000509	3.892546	2.550333	1.0803696829
29	0.4208	0.000404	4.134313	1.643469	1.1699459877
30	0.4214	0.00039	4.040455	1.593103	1.3342500603

Obs	L	a	b
21	33.773333333	6.45	15.686666667
22	33.16	6.88	14.963333333
23	34.136666667	6.52	15.793333333
24	34.18	6.9166666667	16.246666667
25	30.88	6.9766666667	12.74
26	32.38	7.24	14.73
27	29.2	6.5266666667	10.716666667
28	30.99	7.72	12.746666667
29	39.06	7.34	22.08
30	39.605	7.1966666667	22.096666667

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
31	2	35	Soy Flour	12.924634421	7564.8519458	0.2306074766	2.13
32	2	35	Soy Flour	19.606299213	6086.1467601	0.1839130435	1.72
33	2	45	Soy Flour	18.155230596	5560.5713837	0.1658823529	1.3
34	2	45	Soy Flour	21.293588301	6152.0343068	0.184141791	1.11
35	2	55	Soy Flour	20.31496063	4669.4456328	0.13514377	0.48
36	2	55	Soy Flour	20.416197975	5002.4590269	0.6854166667	0.46
37	3	28	Defatted Soy Flour	27.696078431	3335.2477012	0.1330188679	3.08
38	3	28	Defatted Soy Flour	25.784313725	9080.9516266	0.1448630137	3.1
39	3	35	Defatted Soy Flour	25.735294118	3973.1091142	0.0787709497	1.29
40	3	35	Defatted Soy Flour	26.568627451	5150.4232163	0.0997641509	1.23

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
31	0.3805	0.000303	3.747235	1.694815	1.2785200813
32	0.3866	0.000328	3.882016	1.887671	1.4358215604
33	0.3401	0.000302	4.004282	1.815288	1.5841417376
34	0.3427	0.000268	3.860312	1.820393	1.4614900746
35	0.3062	0.000391	3.97514	2.072122	1.5855161835
36	0.3098	0.000343	3.904468	1.911495	1.6755076432
37	0.4716	0.000873	4.587783	2.243348	0.3899108164
38	0.4758	0.000863	4.767172	2.023841	0.5290092114
39	0.4084	0.000399	4.341702	1.928938	0.4784554466
40	0.4011	0.000396	4.091406	1.842579	0.6739372001

Obs	L	a	b
31	39.656666667	7.1266666667	21.906666667
32	40.493333333	6.73	22.63
33	38.366666667	7.5366666667	21.126666667
34	38.15	7.3	20.713333333
35	37.686666667	7.4266666667	20.286666667
36	39.516666667	7.5033333333	22.106666667
37	36.19	12.576666667	36.97
38	36.66	12.683333333	36.976666667
39	36.56	12.646666667	36.236666667
40	36.53	12.89	36.3

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
41	3	45	Defatted Soy Flour	23.62745098	5348.5503925	0.1095854922	0.59
42	3	45	Defatted Soy Flour	27.696078431	4743.3103458	0.1777310924	0.55
43	3	55	Defatted Soy Flour	27.401960784	4636.5810207	0.2073529412	0.35
44	3	55	Defatted Soy Flour	24.509803922	2147	0	0.38
45	3	28	Soy Flour	23.970588235	4386.3515721	0.4217948718	2.24
46	3	28	Soy Flour	20.441176471	-666.6827165	0.329	2.31
47	3	35	Soy Flour	26.078431373	4046.1584285	12.3375	1.43
48	3	35	Soy Flour	20.833333333	4277.0440493	0.397983871	1.47
49	3	45	Soy Flour	28.529411765	4609.1359675	0.2115	0.44
50	3	45	Soy Flour	19.215686275	2791.3949924	0.2583769634	0.49

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
41	0.3516	0.000393	4.505687	2.136481	0.5912548351
42	0.3502	0.000346	3.938078	2.099817	0.7071226536
43	0.2966	0.000463	4.227577	2.344286	0.8665821051
44	0.2945	0.000515	4.331297	2.639371	0.9085379262
45	0.4466	0.000452	4.125219	1.722684	1.1135616675
46	0.4439	0.00046	4.266844	1.719245	1.174391825
47	0.3878	0.000326	3.925188	1.82386	1.2599662354
48	0.3831	0.000373	4.022125	1.936239	1.274373696
49	0.3027	0.000335	3.958813	2.07636	1.3758975813
50	0.2984	0.00026	4.065891	1.712744	1.3522214689

Obs	L	a	b
41	32.233333333	13.666666667	35.983333333
42	32.243333333	13.833333333	35.476666667
43	26.643333333	15.746666667	35.5
44	26.723333333	15.456666667	35.556666667
45	44.81	12.06	43.203333333
46	44.786666667	12.036666667	43.216666667
47	45.216666667	11.896666667	43.353333333
48	45.273333333	11.73	43.386666667
49	44.916666667	11.8	42.676666667
50	44.71	11.766666667	42.58

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
51	3	55	Soy Flour	25.735294118	2846.9281459	0.2902941176	0.33
52	3	55	Soy Flour	26.078431373	3165.4576957	0.4078512397	0.31
53	5	28	Defatted Soy Flour	10.706984668	3731.8787638	0.088125	3.81
54	5	28	Defatted Soy Flour	10.093696763	8056.1449519	0.17625	3.72
55	5	35	Defatted Soy Flour	9.531516184	5067.9754433	0.088125	1.04
56	5	35	Defatted Soy Flour	11.192504259	1534.7236403	0.1175	1.16
57	5	45	Defatted Soy Flour	10.681431005	3064.5547583	0.17625	0.39
58	5	45	Defatted Soy Flour	10.553662692	2302.6362465	0.088125	0.5
59	5	55	Defatted Soy Flour	11.218057922	1689.0874336	0.17625	0.21
60	5	55	Defatted Soy Flour	9.5570698467	405.43339906	0.17625	0.34

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
51	0.2697	0.000332	4.097125	2.015027	1.5139495769
52	0.2673	0.000396	3.889219	2.24593	1.4498705695
53	0.4483	0.000764	3.519608	1.954413	0.7936330959
54	0.4471	0.000698	3.590391	1.876395	0.8216217713
55	0.3173	0.000349	3.111033	1.849638	0.8530276457
56	0.3167	0.000302	5.24589	1.899162	0.925422558
57	0.2301	0.000357	5.187157	2.210761	0.8831480124
58	0.2315	0.000419	5.038345	2.407889	0.9044051291
59	0.2135	0.000453	5.107577	2.589276	0.9509793119
60	0.2146	0.000525	5.311594	2.783789	0.8421681243

Obs	L	a	b
51	41.83	13.836666667	43.686666667
52	41.661666667	13.936666667	43.643333333
53	40.906666667	9.0533333333	27.06
54	40.333333333	9.46	26.646666667
55	38.326666667	8.97	24.763333333
56	38.41	8.9466666667	24.763333333
57	37.773333333	9.3533333333	24.59
58	37.426666667	9.37	24.566666667
59	34.973333333	10.07	21.546666667
60	34.833333333	10.12	21.47

Obs	week	temperature	ingredient	protein	Lysine	Fat	Moisture
61	5	28	Soy Flour	8.6371379898	-1023.32857	0.1768690416	3.54
62	5	28	Soy Flour	10.34923339	-1035.549568	0.2125628141	3.61
63	5	35	Soy Flour	8.8926746167	-1007.998069	0.2125628141	1.08
64	5	35	Soy Flour	9.7103918228	2852.9658999	0.2125628141	1.42
65	5	45	Soy Flour	10.451448041	2998.5529296	0.1783004552	0.42
66	5	45	Soy Flour	9.9659284497	1693.880308	0.2281553398	0.46
67	5	55	Soy Flour	10.63032368	1906.705431	0.177224736	0.34
68	5	55	Soy Flour	8.7904599659	1839.4587673	0.224403183	0.32

Obs	Water_Activity	Delta_Energy	Compressive_extension	Compressive_load	Carotene
61	0.4319	0.000475	4.95578	1.824205	1.190438705
62	0.4322	0.000479	4.788437	1.742216	1.2945291001
63	0.3079	0.000272	4.576688	1.726317	1.0970686326
64	0.3069	0.000252	4.730157	1.667266	1.2002700149
65	0.2458	0.000309	4.645656	2.037408	1.1969981457
66	0.246	0.00032	5.002235	2.094462	1.2973511917
67	0.2132	0.000373	4.619031	2.051247	1.3914409953
68	0.2135	0.00039	4.965501	2.260257	1.3738661501

Obs	L	a	b
61	48.519	9.61	35.2
62	48.453333333	9.7366666667	35.146666667
63	47.573333333	8.7033333333	34.646666667
64	47.45	8.9866666667	34.6
65	46.39	9.77	33.69
66	46.353333333	9.8133333333	33.53
67	43.64	11.166666667	31.106666667
68	43.613333333	11.506666667	31.15

Class Level Information		
Class	Levels	Values
ingredient	2	Defatted Soy Flour Soy Flour

Number of Observations Read	68
Number of Observations Used	68

Dependent Variable: protein protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	275.420567	275.420567	9.72	0.0027
Error	66	1869.191634	28.321085		
Corrected Total	67	2144.612201			

R-Square	Coeff Var	Root MSE	protein Mean
0.128424	30.65653	5.321756	17.35929

Source	DF	Type I SS	Mean Square	F Value	Pr > F
week	1	275.4205671	275.4205671	9.72	0.0027

Source	DF	Type III SS	Mean Square	F Value	Pr > F
week	1	275.4205671	275.4205671	9.72	0.0027

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	20.66861856	1.24202615	16.64	<.0001
week	-1.27860423	0.41000854	-3.12	0.0027

Class Level Information		
Class	Levels	Values
ingredient	2	Defatted Soy Flour Soy Flour

Number of Observations Read	68
Number of Observations Used	68

Dependent Variable: Lysine Lysine

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	100624892.7	33541630.9	9.06	<.0001
Error	64	236948468.6	3702319.8		
Corrected Total	67	337573361.3			

R-Square	Coeff Var	Root MSE	Lysine Mean
0.298083	46.25220	1924.141	4160.108

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	20297939.37	20297939.37	5.48	0.0223
temperature	1	3713772.12	3713772.12	1.00	0.3203
week	1	76613181.18	76613181.18	20.69	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	20297939.37	20297939.37	5.48	0.0223
temperature	1	757081.18	757081.18	0.20	0.6527
week	1	76613181.18	76613181.18	20.69	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	5782.522564	B	999.9790953	5.78	<.0001
ingredient Defatted Soy Flour	1092.701438	B	466.6728179	2.34	0.0223
ingredient Soy Flour	0.000000	B	.	.	.
temperature	-10.270997		22.7132008	-0.45	0.6527
week	-679.198573		149.3076235	-4.55	<.0001

Dependent Variable: Water_Activity Water Activity

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.32920338	0.06584068	128.93	<.0001
Error	62	0.03166249	0.00051069		
Corrected Total	67	0.36086587			

R-Square	Coeff Var	Root MSE	Water_Activity Mean
0.912260	6.202280	0.022598	0.364356

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	0.00951380	0.00951380	18.63	<.0001
temperature	1	0.20698525	0.20698525	405.31	<.0001
week	1	0.07001312	0.07001312	137.10	<.0001
temperature*week	1	0.04032645	0.04032645	78.97	<.0001
week*ingredient	1	0.00236475	0.00236475	4.63	0.0353

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	0.00850638	0.00850638	16.66	0.0001
temperature	1	0.00266166	0.00266166	5.21	0.0259
week	1	0.01560948	0.01560948	30.57	<.0001
temperature*week	1	0.04032645	0.04032645	78.97	<.0001
week*ingredient	1	0.00236475	0.00236475	4.63	0.0353

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	0.4497855107	B	0.02056602	21.87	<.0001
ingredient Defatted Soy Flour	0.0430504888	B	0.01054830	4.08	0.0001
ingredient Soy Flour	0.0000000000	B	.	.	.
temperature	-.0011522906		0.00050473	-2.28	0.0259
week	0.0409559996	B	0.00695187	5.89	<.0001
temperature*week	-.0014901827		0.00016770	-8.89	<.0001
week*ingredient Defatted Soy Flour	-.0074930866	B	0.00348213	-2.15	0.0353
week*ingredient Soy Flour	0.0000000000	B	.	.	.

Dependent Variable: Fat Fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3.8385015	1.2795005	0.57	0.6336
Error	64	142.4230914	2.2253608		
Corrected Total	67	146.2615929			

R-Square	Coeff Var	Root MSE	Fat Mean
0.026244	408.0482	1.491764	0.365585

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	3.06990865	3.06990865	1.38	0.2445
temperature	1	0.47254817	0.47254817	0.21	0.6465
week	1	0.29604471	0.29604471	0.13	0.7165

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	3.06990865	3.06990865	1.38	0.2445
temperature	1	0.55856664	0.55856664	0.25	0.6181
week	1	0.29604471	0.29604471	0.13	0.7165

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	0.8216734498	B	0.77527213	1.06	0.2932
ingredient Defatted Soy Flour	-.4249504231	B	0.36180599	-1.17	0.2445
ingredient Soy Flour	0.0000000000	B	.	.	.
temperature	-.0088222401		0.01760928	-0.50	0.6181
week	0.0422205291		0.11575646	0.36	0.7165

Dependent Variable: Moisture Moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	73.94009365	24.64669788	62.09	<.0001
Error	64	25.40438897	0.39694358		
Corrected Total	67	99.34448263			

R-Square	Coeff Var	Root MSE	Moisture Mean
0.744280	36.34789	0.630035	1.733346

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	2.14153759	2.14153759	5.40	0.0234
temperature	1	64.12883144	64.12883144	161.56	<.0001
week	1	7.66972462	7.66972462	19.32	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	2.14153759	2.14153759	5.40	0.0234
temperature	1	58.07762533	58.07762533	146.31	<.0001
week	1	7.66972462	7.66972462	19.32	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	5.710461954	B	0.32742991	17.44	<.0001
ingredient Defatted Soy Flour	0.354926471	B	0.15280583	2.32	0.0234
ingredient Soy Flour	0.000000000	B	.	.	.
temperature	-0.089959244		0.00743714	-12.10	<.0001
week	-0.214899251		0.04888880	-4.40	<.0001

Dependent Variable: Delta_Energy   Delta Energy

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.7551698E-7	4.8775849E-7	29.92	<.0001
Error	65	1.0595609E-6	1.6300936E-8		
Corrected Total	67	2.0350778E-6			

R-Square	Coeff Var	Root MSE	Delta_Energy Mean
0.479351	28.31488	0.000128	0.000451

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	6.39909E-7	6.39909E-7	39.26	<.0001
temperature	1	3.3560797E-7	3.3560797E-7	20.59	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	6.39909E-7	6.39909E-7	39.26	<.0001
temperature	1	3.3560797E-7	3.3560797E-7	20.59	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	0.0006254923	B	0.00006373	9.81	<.0001
ingredient Defatted Soy Flour	0.0001940147	B	0.00003097	6.27	<.0001
ingredient Soy Flour	0.0000000000	B	.	.	.
temperature	-.0000067897		0.00000150	-4.54	<.0001

Dependent Variable: Compressive_extension Compressive extension

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.09173378	0.69724459	1.17	0.3298
Error	64	38.28437755	0.59819340		
Corrected Total	67	40.37611133			

R-Square	Coeff Var	Root MSE	Compressive_extension Mean
0.051806	17.25142	0.773430	4.483281

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	0.58601787	0.58601787	0.98	0.3260
temperature	1	1.49728564	1.49728564	2.50	0.1186
week	1	0.00843027	0.00843027	0.01	0.9059

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	0.58601787	0.58601787	0.98	0.3260
temperature	1	1.50272542	1.50272542	2.51	0.1179
week	1	0.00843027	0.00843027	0.01	0.9059

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	3.830071446	B	0.40195253	9.53	<.0001
ingredient Defatted Soy Flour	0.185665397	B	0.18758424	0.99	0.3260
ingredient Soy Flour	0.000000000	B	.	.	.
temperature	0.014470422		0.00912982	1.58	0.1179
week	-0.007124685		0.06001583	-0.12	0.9059

Dependent Variable: Compressive_load Compressive load

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3.49455253	1.16485084	43.80	<.0001
Error	64	1.70201764	0.02659403		
Corrected Total	67	5.19657017			

R-Square	Coeff Var	Root MSE	Compressive_load Mean
0.672473	8.489571	0.163077	1.920907

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	1.01115983	1.01115983	38.02	<.0001
temperature	1	1.68625555	1.68625555	63.41	<.0001
week	1	0.79713715	0.79713715	29.97	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	1.01115983	1.01115983	38.02	<.0001
temperature	1	1.39921577	1.39921577	52.61	<.0001
week	1	0.79713715	0.79713715	29.97	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	1.061123280	B	0.08475123	12.52	<.0001
ingredient Defatted Soy Flour	0.243885199	B	0.03955192	6.17	<.0001
ingredient Soy Flour	0.000000000	B	.	.	.
temperature	0.013963160		0.00192501	7.25	<.0001
week	0.069280584		0.01265427	5.47	<.0001

Dependent Variable: Carotene Carotene

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	7.55319699	1.88829925	69.10	<.0001
Error	63	1.72166465	0.02732801		
Corrected Total	67	9.27486165			

R-Square	Coeff Var	Root MSE	Carotene Mean
0.814373	14.38386	0.165312	1.149287

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	5.62443393	5.62443393	205.81	<.0001
temperature	1	0.90399195	0.90399195	33.08	<.0001
week	1	0.87282254	0.87282254	31.94	<.0001
week*ingredient	1	0.15194858	0.15194858	5.56	0.0215

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	2.45026742	2.45026742	89.66	<.0001
temperature	1	1.11378867	1.11378867	40.76	<.0001
week	1	0.87282254	0.87282254	31.94	<.0001
week*ingredient	1	0.15194858	0.15194858	5.56	0.0215

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	1.203935237	B	0.09201992	13.08	<.0001
ingredient Defatted Soy Flour	-0.730655286	B	0.07716312	-9.47	<.0001
ingredient Soy Flour	0.000000000	B	.	.	.
temperature	0.012457841		0.00195140	6.38	<.0001
week	-0.102527130	B	0.01807657	-5.67	<.0001
week*ingredient Defatted Soy Flour	0.060064280	B	0.02547252	2.36	0.0215
week*ingredient Soy Flour	0.000000000	B	.	.	.

Dependent Variable: L L

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1481.318917	740.659458	67.25	<.0001
Error	65	715.890177	11.013695		
Corrected Total	67	2197.209094			

R-Square	Coeff Var	Root MSE	L Mean
0.674182	8.296189	3.318689	40.00257

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	1327.545689	1327.545689	120.54	<.0001
temperature	1	153.773228	153.773228	13.96	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	1327.545689	1327.545689	120.54	<.0001
temperature	1	153.773228	153.773228	13.96	0.0004

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	50.23448422	B	1.65666065	30.32	<.0001
ingredient Defatted Soy Flour	-8.83690686	B	0.80490025	-10.98	<.0001
ingredient Soy Flour	0.00000000	B	.	.	.
temperature	-0.14533649		0.03889563	-3.74	0.0004

Dependent Variable: a a

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	17.1899053	5.7299684	1.19	0.3191
Error	64	307.1044489	4.7985070		
Corrected Total	67	324.2943542			

R-Square	Coeff Var	Root MSE	a Mean
0.053007	21.97454	2.190549	9.968578

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	0.17368644	0.17368644	0.04	0.8497
temperature	1	12.24970962	12.24970962	2.55	0.1150
week	1	4.76650928	4.76650928	0.99	0.3227

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	0.17368644	0.17368644	0.04	0.8497
temperature	1	10.33484105	10.33484105	2.15	0.1471
week	1	4.76650928	4.76650928	0.99	0.3227

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	7.961624702	B	1.13843181	6.99	<.0001
ingredient Defatted Soy Flour	0.101078431	B	0.53128629	0.19	0.8497
ingredient Soy Flour	0.000000000	B	.	.	.
temperature	0.037948383		0.02585797	1.47	0.1471
week	0.169412417		0.16998010	1.00	0.3227

Dependent Variable: b b

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1132.023201	1132.023201	19.10	<.0001
Error	66	3911.773265	59.269292		
Corrected Total	67	5043.796466			

R-Square	Coeff Var	Root MSE	b Mean
0.224439	26.50931	7.698655	29.04132

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ingredient	1	1132.023201	1132.023201	19.10	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ingredient	1	1132.023201	1132.023201	19.10	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	33.12144608	B	1.32030847	25.09	<.0001
ingredient Defatted Soy Flour	-8.16024510	B	1.86719815	-4.37	<.0001
ingredient Soy Flour	0.00000000	B	.	.	.