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Determination of Beta-Carotene Content and Consumer Acceptability of Sweet Potato Cookies by Adults and Preschool Children

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Determination of beta-carotene content and consumer acceptability of sweet potato cookies by adults and preschool children

By

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Vitamin A deficiency is recognized as a major health concern worldwide, especially in developing countries. Sweet potatoes are a cash crop that is abundantly grown and available, providing an excellent source of the carotenoid, beta-carotene. Carotenoids are precursors to vitamin A (retinol). Three sweet potato cookie products were developed: gluten-free, wheat-containing, and gluten-free with extra sweet potato. Products were evaluated by adults and pre-school aged children based on appearance, aroma, texture, flavor, and overall acceptability. Results showed that overall the children liked both the gluten-free and wheat-containing cookies ($p<0.05$). Adults preferred ($p<0.05$) the gluten-free with extra sweet potato and the wheat-containing products. The gluten-free cookie contained 10.1 parts per million of beta-carotene as determined by high-performance liquid chromatography.

Key words: sweet potato, vitamin A, beta-carotene, cookies, gluten-free

DEDICATION

This thesis is dedicated to my family, whether blood or adopted, close family friends and friends who have showed undying support in the pursuit of furthering my education.

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TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER

I. INTRODUCTION 1

II. REVIEW OF LITERATURE 4

Vitamin A 4
Vitamin A Degradation 9
Anti-Oxidative Properties ('Quencher') 12
Isomers of Beta-Carotene 13
Vitamin A Activity 16
Vitamin A Requirements and Deficiency 17
Sweet Potatoes (*Ipomoea batatas*) 24
Sensory Analysis 26
High-Performance Liquid Chromatography 27

III. MATERIALS AND METHODS 30

Sweet Potato Cookie Preparation 30
Development of Beta-Carotene Standard Curve 32
Determination of Beta-Carotene Content 33
Sensory Evaluation 34
Sensory Evaluation by Adults 35
Sensory Evaluation by Pre-School Aged Children 36
Institutional Review Board Approval 37
Statistical Analysis 38

IV. RESULTS AND DISCUSSION 39

Beta-Carotene Content 39
Sensory Evaluation 39

Adults 39
Pre-School Aged Children 43
Limitations 44

V. CONCLUSION 46

REFERENCES 49

APPENDIX

A. SCORE SHEETS FOR CONSUMER SENSORY PANELS 53

B. INFORMED CONSENT AND MISSISSIPPI STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD APPROVAL 58

LIST OF TABLES

2.1 Retinol Activity Equivalents (RAE) Ratios for Beta-Carotene and Other Provitamin A Carotenoids 17

3.1 Gluten-Free Sweet Potato Cookies Recipe 32

4.1 Mean Scores for Consumer Acceptability (n=148) of Three Sweet Potato Cookie Samples[1] 41

4.2 Mean Scores for Overall Consumer Acceptability of Sweet Potato Cookie Samples According to Different Clusters of Consumer Segments Using a Hedonic Scale[1] 42

4.3 Mean Scores for Children's Overall Acceptability of Two Sweet Potato Cookie Samples[1] 44

LIST OF FIGURES

2.1 Chemical Structure of All-*Trans*-Retinol....4

2.2 Chemical Structures of Retinoids....5

2.3 Configurations of 1,2-dichloroethane 15

2.4 Configurations of 1,2-dichloroethane 15

2.5 Category of Public Health Significance (Prevalence of Serum Retinol less than 0.70µmol/l). Source: Childinfo.org, 2009. 19

CHAPTER I

INTRODUCTION

The World Health Organization (WHO) indicates that vitamin A deficiency remains a significant public health problem at a global level (World Health Organization, 2009). In the United States, vitamin deficiencies are not a major concern today because many of the commonly consumed foods are fortified. Unless people are not eating enough kilocalories on a daily basis that may deplete vitamin stores, or taking medications that inhibit absorption or accelerate secretion, Americans are generally safe from vitamin deficiencies. Vitamin/mineral supplements are readily available in the marketplace for those identified as having a deficiency. However, developing countries are not as fortunate to have the many preventative measures that are available to industrialized countries.

Prevalence of vitamin A deficiency in developing countries is steadily rising, especially in Sub-Saharan Africa and Southeast Asia, in which 30.3% and 82.4%, respectively, of pre-school children are thought to suffer from night blindness as a result of being vitamin A deficient (VAD), attributing to high rates of deaths in children under the age of five years (World Health Organization, 2009). Fruits and vegetables are the main vitamin A food sources for developing countries, which may provide inadequate dietary intake of vitamin A. Adequate vitamin intake during childhood is needed for growth and recovering from illness and loss of nutrients during infections that

specifically occur during childhood. Vitamin A deficiency is the leading cause of childhood blindness (Kello and Gilbert, 2003; Muhit *et al*., 2007; World Health Organization, 2009).

A child deficient in vitamin A has a 23% greater risk of dying from diseases such as the measles, malaria, or diarrhea (not a disease, but a symptom) before blindness is diagnosed. To make matters worse, 5.2 million pre-school aged children are diagnosed as clinically VAD. The WHO reports that a total of 45 countries have a clinical vitamin A deficiency problem, whereas a total of 122 countries have a sub-clinical problem. There are three different deficiency control strategies: supplementation, food fortification, and dietary diversity. International supplementation strategies to control VAD are currently high-dose vitamin A supplements, administered every four to six months for all children aged six months to five years of age that live in deficient areas. This means of supplementation is cost-effective, safe, and efficient in order to improve childhood survival and eradicate VAD (World Health Organization, 2009).

More countries are trying to implement food fortification to assist in the control of VAD (World Health Organization, 2009). Although mandated food fortification can take years to incorporate, some food products that have been used as vitamin A carriers thus far include: sugar, oil, milk, margarine, infant foods, and various types of flour. For example, Latin America has supported the effort to supplement sugar with vitamin A, targeting supplementation towards children ages six to 24 months and/or to high risk areas (World Health Organization, 2009). Ramakrishnan and Darnton-Hill (2002) reported that positive results were presented from efficacy trials testing oil and sugar fortified with vitamin A in Central America. Vitamin A consumption that occurs in

developing countries usually comes from fruits and vegetables, accounting for 80% of vitamin A intake. The WHO recommends the promotion of other bioavailable vitamin A food sources that are of animal origin in addition to fruit and vegetable consumption (World Health Organization, 2009).

Either strategy that is used to control VAD or any combination of the three strategies, the proposed outcome is the same; adequate vitamin A supplementation to improve the quality of life in young children. Exploring vitamin A food sources, including beta-carotene, and investigating the utilization of sweet potatoes (a well known source of vitamin A) in the unique form of a cookie, may improve the intake of vitamin A in children through the consumption of sweet potato cookies. The purpose of this study was to develop an acceptable sweet potato cookie, conduct sensory analysis to determine consumer acceptability of the cookie, and determine the beta-carotene content of the cookie.

CHAPTER II

REVIEW OF LITERATURE

Vitamin A

Vitamin A is a fat-soluble vitamin, whose primary well-known function is vision. The term vitamin A is an umbrella term for compounds that contain the biological activity of retinol or contain the retinol-based structure (Figure 2.1). The plural term, retinoids, which are structurally similar, include retinol, retinal, retinoic acid, retinyl ester and synthetic analogues. Retinoids contain three major components: β-Ionone ring, a polyunsaturated side chain and one of four identifying groups (Helwig, n.d.).

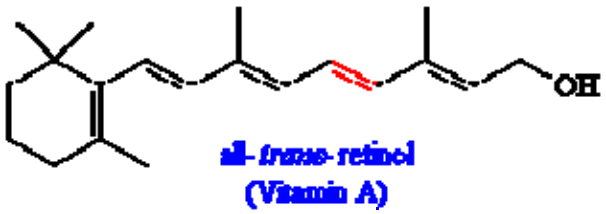

Figure 2.1 Chemical Structure of All-*Trans*-Retinol

The β-Ionone ring serves as an aromatic compound, with a violet smell (Farlex, n.d.). The polyunsaturated side chains are characterized by five conjugated double bonds which are alternating single and double bonds, enabling the electrons to be "delocalized to move around the whole system" (Gregory III, 2008). In simple terms, they can be

broken down by the body more easily to be distributed throughout the body for various functions. The retinoids are categorized according to the type of isoform side chains they have at the end of their structure, either an alcohol, aldehyde, carboxylic, or ester group; thus, giving them their names: retinol (alcohol), retinal (aldehyde), retinoic acid (carboxylic) and retinyl ester (ester) (Figure 2.2). All of these characteristics are essential for the activity of vitamin A (Helwig, n.d.).

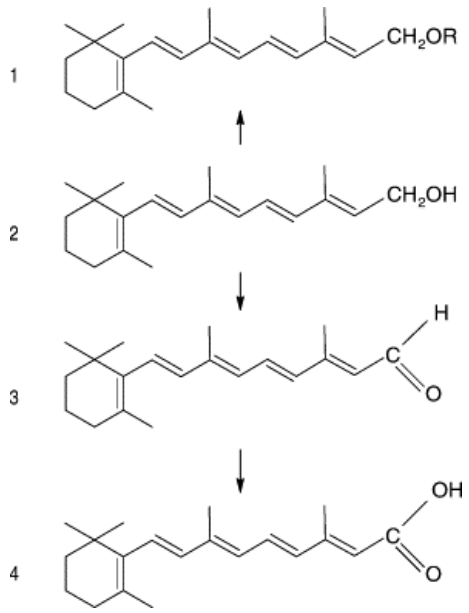

Figure 2.2 Chemical Structures of Retinoids

Notes: (1) Retinyl ester, (2) Retinol, (3) Retinal and (4) Retinoic acid.
Source: Merris *et al*., 2002

Retinol is recognized as preformed vitamin A, signifying that it is the primary form of vitamin A utilized in the body (Gropper *et al*., 2009). It is described as being a "pale yellow crystalline solid" (Helwig, n.d.). The majority of the retinoids convert into retinol once absorbed as well as individually having their own functions. The efficiency of vitamin A absorption is 70-90%. The major body pool or concentration of vitamin A is

located in the liver (Gropper *et al*., 2009). More than 90 percent of the retinol stored within the body is in the form of retinyl esters due to the re-esterification of retinol before it is stored. The absorption process of vitamin A only takes two to six hours to be completed after digestion (Helwig, n.d.).

Most importantly, in order for optimal absorption of vitamin A, the body needs at least 10g of dietary fat or more, not only because it is a fat-soluble vitamin, but for adequate digestion and absorption. The enzyme pepsin secreted from the stomach and proteases/lipases in the duodenum/intestines are needed to release retinyl esters and carotenes from tightly bound proteins. Bile, produced by the liver and stored in the gallbladder, is needed for emulsification in the duodenum, forming mixed micelle structures to protect the non-polar or hydrophobic contents from the polar or hydrophilic compounds (i.e., triglycerides, esters, carotenoids) (Gropper *et al*., 2009). They are then passed through the brush border and absorbed into enterocytes, where they are converted into the active forms of vitamin A. The newly formed products are then further packaged into chylomicrons, which carry the micelle structures through the lymphatic system and eventually into general circulation to be distributed to the designated organs (i.e., liver) (Helwig, n.d.). In foods, vitamin A is primarily in the form of retinyl esters. However, retinoids that are exposed to light, heat, or some metals lose their vitamin potency through oxidation (Gropper *et al*., 2009).

Retinal and retinoic acid are two byproducts of retinol that play roles in vision (retinal) and intracellular messaging that affects transcription of a number of genes (retinoic acid). Vitamin A is also essential for cell differentiation, growth, reproduction, bone development and immune system actions. Vitamin A is known for visual ability

through the cleaving of rhodopsin (pigment of photoreceptor cells) when a flash of light hits the dark-adapted retina. This cleaving releases vitamin A, sending signals to the brain that promote eye sight. In order to regain proper eye sight in the dark, rhodopsin must be recreated from vitamin A. When there is a failure or delayed vision regain, it is known as night blindness. This is one of many conditions that occur when vitamin A deficiency ensues. Cell differentiation is primarily promoted by retinoic acid, which helps maintain the normal structure and functions of epithelial cells. For example, retinoic acid aids in the differentiation of keratinocytes (immature skin cells) to epidermal cells. Although it is not completely understood how vitamin A stimulates growth, it is known that growth factors that bind to specific receptors on cell surfaces stimulate cell growth (Gropper *et al*., 2009).

Retinoic acid also seems to increase the number of cell receptors for growth factors. As mentioned previously, vitamin A aids in intracellular messaging also known as "cell to cell communication" through gap junctions or cell to cell channels. These channels exchange small signaling compounds creating communication through cells. Vitamin A not only stimulates the production of specific gap junction proteins, but also plays a role in controlling cell growth. Uncontrolled cell growth results from a lack of gap junction communication. Cell surfaces may also be modified through retinoic acid and retinol by either increasing glycoprotein synthesis at the gene level or enhancing the joining of glycoproteins to cell surfaces promoting cell adhesion. Vitamin A is known to improve skin structure and aids in decreased severity and frequency of diseases such as the measles (Gropper *et al*., 2009).

Carotenoids are the precursor to vitamin A (α-carotene, β-cryptoxanthin and β-carotene), otherwise known as provitamin A carotenoids. The most common and potent carotenoid is β-carotene. These provitamins can be converted to retinol in the body through cleaving C15-C15' bonds, creating two molecules of retinal (Gropper *et al*., 2009). They cannot be synthesized in animals or humans and therefore must be obtained through dietary intake. Bacteria, algae, fungi and plants have the ability to synthesize carotenoids (Gropper *et al*., 2009). β-carotene is found in yellow, orange, and green leafy fruits and vegetables. The more color intensity of the fruit or vegetable, the more β-carotene it possesses. Sources of carotenoids include carrots, pumpkin, winter squash, sweet potatoes, spinach, collard greens, kale, cantaloupe, apricots, mangoes, and nectarines (Drake *et al*., 2009). Other sources of vitamin A include animal products such as liver, butter, margarine, milk, cod liver oil, cheese, eggs and fortified ready to eat cereals (Krinke, 2008).

Vitamin E also protects carotene and its products from oxidation. The efficiency of carotenoid absorption is less than 5% (uncooked vegetables) to 60% (juices). Contrary to the previous statement, fiber and vitamin E consumption can also reduce the absorption of carotenoids. As with retinoids, the requirement for optimal absorption is 10g of fat or more, enzymes secreted from the intestines for carotenoids to release from proteins, bile, and mixed micelle formations (Gropper *et al*., 2009).

Carotenoids are also believed to have anti-oxidative properties which can protect against cancer and other diseases (Gropper *et al*., 2009). There are three different types of oxygenated carotenoids: cantha-xanthin, lutein, and zea-xanthin. These are not precursors of vitamin A but rather successors of carotenoids that play important roles in vision.

Lutein is found in a small area of the retina called the macula and in food sources such as egg yolks, spinach, and kale. The macula is necessary for providing central vision. Zeaxanthin is similar to lutein in the fact that both are found in the macula of the eye (Drake *et al*., 2009). The differentiating factor between the two is where the double bond lies in one of the end rings (Gropper *et al*., 2009). Carotenoids have similar biological functions as retinoids, participating in cell proliferation, growth, differentiation, enhancement of cell mediated immune functions and anti-oxidative properties (Gropper *et al*., 2009).

Vitamin A Degradation

Vitamin A is a fat-soluble vitamin that is vital for vision, immunity, growth, reproduction, and other functions. However, vitamin A is somewhat unstable, undergoing oxidation in three different ways: auto-oxidation, thermal isomerization, and photochemical isomerization (Gregory III, 2008). Also, it is important to note that during the cleaving of beta-carotene to produce retinal, it can undergo oxidation, partially diminishing vitamin A activity (Gropper *et al*., 2009). However, according to Drake *et al*. (2009), it is believed that a high amount of vitamin A stored in the liver and intestines limits the ability for cleavage.

"Auto," the prefix in auto-oxidation, means "self-acting," alluding to the fact that the entire process is promoted by free radicals which are self-formulated from the lipid oxidation that occurs when unsaturated fatty acids are exposed to oxygen. There are three steps in auto-oxidation: initiation, propagation, and termination (McClements and Decker, 2008). The process of initiation occurs in the presence of oxygen or UV radiation. In initiation, a hydrogen molecule is extracted from a fatty acid, which forms a free radical also known as an alkyl radical. Free radicals are molecules or atoms

containing unpaired electrons, especially a hydroxyl radical (-OH) which has very high energy and oxidizes any molecule through hydrogen extraction. Also, the higher the degree of unsaturation in the fatty acid, the easier it is to form fatty acid radicals. The alkyl radical then delocalizes the double bonds of the fatty acid, shifting them to different locations on the fatty acid structure, forming alternating double bonds called conjugated bonds. *Cis* or *trans* bonds are formed which ultimately have an effect on vitamin A activity. From this hydrogen extraction and delocalizing of double bonds, the energy of the carbon-hydrogen bonds is steadily decreasing, allowing for further hydrogen extraction, radical formation, and faster oxidation rates (McClements and Decker, 2008).

In propagation, the alkyl radical interacts with the oxygen either in the form of singlet oxygen or triplet oxygen. Singlet oxygen is an excited form of oxygen that has two electrons orbiting in opposite directions. Triplet oxygen is a bi-radical that is of low energy, and has two electrons orbiting in the same direction, which according to the Pauli exclusion principle cannot occur in the same electron orbital. When the alkyl radical reacts with the triplet oxygen, it forms a covalent bond and will not induce hydrogen extraction. However, when the alkyl radical interacts with the singlet oxygen, it results in a peroxyl radical. Because the spin direction of the singlet oxygen matches that of the electrons on the double bonds, it reacts with unsaturated fatty acids 1,500 times faster than that of triplet oxygen. This newly formed radical then allows for further hydrogen extraction from other fatty acids, once again making the fatty acids carbon-hydrogen bonds energy weaker and making it easier for the peroxyl radical to attack. Through the addition of hydrogen's to the peroxyl radical, a hydroperoxide radical or lipid hydroperoxide is formed (McClements and Decker, 2008).

Singlet oxygen can be formed by two different pathways: type 1 and type 2, both are produced by photosensitization. Type 1 involves the absorption of energy from light from photosensitizers that are found in foods, creating an "excited singlet state." The excited singlet state is then converted to an excited triplet state that can interact with unsaturated fatty acids such as the ones found in vitamin A, initiating the first step in auto-oxidation, initiation. Type 2 involves the excited triplet state interacting with the triplet oxygen, ultimately forming singlet oxygen. The type 2 pathway is likely to occur in high-oxygen environments whereas type 1 is likely to occur in the opposite environment (McClements and Decker, 2008).

The termination step refers to the formation of a non-radical species through the conjunction of two radicals. Specifically, this reaction will ensue in the presence of low oxygen environments forming fatty acid dimers. This process of auto-oxidation through the steps of initiation and propagation is known as primary oxidation. The products produced from primary oxidation are not only the first to occur but also are manifested early within the lipid oxidation process. However, they are not considered volatile in the fact that they do not exhibit any off-flavors or aromas (McClements and Decker, 2008). Secondary oxidation however, results from the breakdown of the hydroperoxides, exhibits volatile off-flavors and aromas as well as non-volatiles such as the ones that can be experienced when smelling or tasting rancid oil. Some pro-oxidants that promote the breakdown of lipid hydroperoxides are as follows: thermal processing/conditions, transition metals associated with biological materials, water, light, ingredients and packaging materials, and transition metals associated with protein (i.e., iron in myoglobin, hemoglobin, peroxidases, and catalases). Although, β-scission is the general

way in which hydroperoxides are broken down. In the reaction of β-scission, carbons are broken apart from each other on the fatty acid chain, forming several open chains that produce aldehydes and radicals such as the alkyl radical. It is important to keep in mind that the secondary products of aldehydes, ketones, and alcohols due to the dissemination of lipid hydroperoxides are the cause of these off-flavors and aromas, not the primary products of peroxyl and hydroperoxide radicals (McClements and Decker, 2008).

Thermal isomerization refers to the conversion of a compound into an isomer in the presence of heat. An isomer is two or more compounds with the same formula but different arrangement of atoms in the molecule such as all-*trans*, 9-*cis*-β-carotene and 13-*cis*-β-carotene that are all isomers of β-carotene. In thermal isomerization, in the presence of light, acid, chlorinated solvents and dilute iodine, all-*trans* forms of carotenoids are converted to the different *cis* isomers (Gregory III, 2008). Photochemical isomerization refers to the same process as thermal isomerization, only in the presence of light or substances that make an organism, cell or tissue sensitive to light known as photo-sensitizers (i.e., chlorophyll, riboflavin and myoglobin) (Gregory III, 2008).

Anti-Oxidative Properties ('Quencher')

Despite vitamin A's inability to stabilize in the presence of high-oxygen environments, metals, and light, it still has the ability to serve as an anti-oxidative force in itself and food products in low-oxygen environments. According to McClements and Decker (2008), there are two different ways in which singlet oxygen (the main cause of lipid oxidation) can be 'quenched' or controlled: chemically and physically. Singlet oxygen is chemically quenched by carotenoids when the double bonds of the carotenoid

are altered by the singlet oxygen. Oxygenated carotenoids are formed and broken down into aldehydes, ketones, and endoperoxides resulting in a loss of color through carotenoid breakdown (McClements and Decker, 2008).

Physical quenching is a more ideal method to control singlet oxygen. In this type of quenching, energy is transferred from the singlet oxygen to the carotenoid, creating an "excited state carotenoid," and solidifying a triplet oxygen state. The energy from the excited carotenoid eventually becomes unavailable or lost via interactions (i.e., vibrations or rotations) with the medium or solvent returning the carotenoid to its original state. The nine or more conjugated bonds and the β-ionone ring are essential characteristics of carotenoids that serve as necessary components to assist in the carotenoids anti-oxidative abilities. It is also possible for carotenoids to physically absorb energy from photosensitizers in the prevention of singlet oxygen formation. It is important to note that vitamin A's anti-oxidative properties are only effective in low-oxygen environments (McClements and Decker, 2008).

Isomers of Beta-Carotene

In the vitamin A degradation section, it was discussed how isomerization of vitamin A or β-carotene can alter the ratios, quantities, and vitamin A activity of the specific isomers found in β-carotene. There are two forms of isomers of β-carotene: *trans* and *cis*, with *trans* described as more stable than *cis*. *Cis* isomers are described as polar, less crystallized and more soluble in oil when compared to *trans*. Various ratios of these isomers are found in food sources, including all-*trans*, 9-*cis*, 13-*cis* and 15-*cis*, although all-*trans*-β-carotene is thought to predominate in nature (Patrick, 2000). Also, all-*trans*

isomers are believed to provide the most vitamin A activity when compared to the other isomers (Gregory III, 2008). For example, raw carrots, tomatoes, and fresh sweet potatoes are believed to consist of 98% of all-*trans* isomers. The latter isomer forms are increased in food products after undergoing heat processes or isomerization which causes vitamin A activity loss. It has been hypothesized that all-*trans* is more easily absorbed in the body than the others and because of this notion, all synthetically produced β-carotene is made in the form of all-*trans*-β-carotene (Patrick, 2000). However, the mechanism for how or why these two isomers (all-*trans* and 9-*cis*) have different pathways and efficiencies of absorption is unknown.

As mentioned earlier, isomers are molecules having the same molecular formula but different arrangements. However, this does not include isomers that are simply arranged differently due to the rotating about a specific bond (single bond) or as a whole structure as shown in Figure 2.3. Instead, *cis*-*trans* bonds refer to an isomer that is restricted to rotating due to the presence of a double bond. The term *cis* refers to "same side" and the term *trans* refers to "across" or opposite sides as shown in Figure 2.4 (Clark, 2012). *Trans* isomers give a better stacking or "packing" effect and are straighter, making their intermolecular forces stronger. Whereas *cis* isomers have a "U" shape and do not pack as effectively as *trans*, making their intermolecular forces weaker and easily broken apart at lower melting points than *trans* (Clark, 2012).

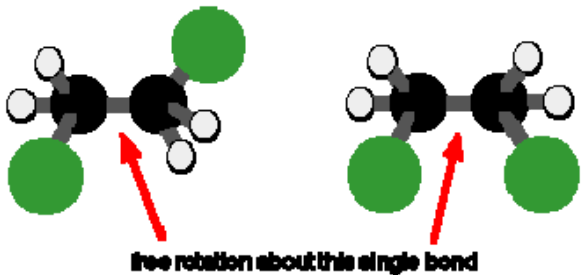

Figure 2.3 Configurations of 1,2-dichloroethane

Source: Clark, 2012

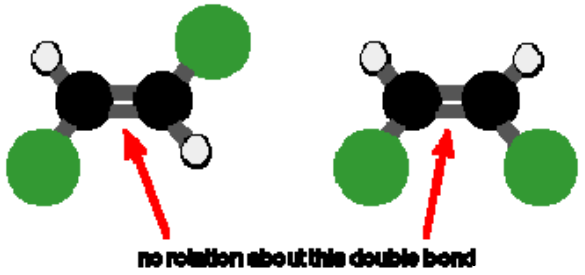

Figure 2.4 Configurations of 1,2-dichloroethane

Notes: Left (*trans*) and right (*cis*)
Source: Clark, 2012

Sixty percent of human tissue consists of the all-*trans* isomer. Twenty-five percent of the liver and 10 percent of the adrenals contain 9-*cis*-β-carotene along with the kidneys, ovaries, testes, and adipose tissue. Although all-*trans* isomers appear to be favored in the human body and in foods, the 9-*cis* isomer is believed to be a direct precursor to the formation of 9-*cis* retinoic acid in the intestinal enterocytes. Retinoic acid is important for hormone signaling, normal reproduction, maintenance of epithelial cells, and preventing carcinogenesis (Patrick, 2000).

Vitamin A Activity

Based on the fact that retinol is the primary form in which vitamin A is utilized in the body and the majority of all other retinoids and carotenoids are converted to retinol, it is assumed that the remainder of the provitamin A compounds or precursors have lesser amounts of vitamin A activity. β-carotene has 50 percent of retinol's vitamin A activity (Gregory III, 2008) or only one-twelfth of the vitamin A activity of retinol from food sources, with α-carotene and β-cryptoxanthin possessing only one-twenty-fourth activity (Drake *et al*., 2009). Also, beta-carotene has two β-ionone rings in the chemical structure, and it is assumed that this assists beta-carotene in the greater overall vitamin A activity that it possesses over the other carotenoids (Aalbersberg, n.d.). Particularly of note is that it would take two micrograms of supplemental β-carotene to render one microgram of retinol (2:1) and twelve micrograms of dietary β-carotene to render one microgram of retinol (12:1) (Drake *et al*., 2007). In general, Table 2.1 showcases the retinol activity equivalents (RAE) ratios for retinol, β-carotene, and other vitamin A-active carotenoids (Gregory III, 2008).

Table 2.1 Retinol Activity Equivalents (RAE) Ratios for Beta-Carotene and Other Provitamin A Carotenoids

Retinol activity equivalents (RAE) ratios for beta-carotene and other provitamin A carotenoids		
Quantity Consumed	Quantity Bioconverted to Retinol	RAE ratio
1 µg of dietary or supplemental vitamin A	1 µg of retinol[a]	1:1
2 µg of supplemental beta-carotene	1 µg of retinol	2:1
12 µg of dietary beta-carotene	1 µg of retinol	12:1
24 µg of dietary alpha-carotene	1 µg of retinol	24:1
24 µg of dietary beta-cryptoxanthin	1 µg of retinol	24:1

[a]One µg of retinol is equivalent to 3.33 IU of retinol.
Source: Drake *et al*., 2007

Vitamin A Requirements and Deficiency

The recommended dietary allowance (RDA) for vitamin A in children ages one to eight years is 300-400µg/day. The vitamin A tolerable upper intake level (UL) for children ages one to eight years is 600-900µg/day. The RDA for vitamin A for pregnant women 18 years old and younger is 750µg/day and 770µg/day for pregnant women 19 years and older. During lactation, the RDA for vitamin A in women 18 years old and younger is 1,200µg/day and 1,300µg/day for those 19 and older. The UL for pregnant and lactating women less than 19 years old is 2,800µg/day and 3,000µg/day for women 19 years and older (Institute of Medicine, Food and Nutrition Board, 2004). Since vitamin A has a recommended UL, it is inferred that vitamin A has toxicity complications in addition to deficiency complications.

Although one focus of the present study is with pre-school aged children, vitamin A status in pregnant and lactating women is also important. The placenta is a round,

disklike shape that is made up of embryonic tissue, and encompasses the fetus in the mother's womb. The primary functions of the placenta are to produce hormones and enzymes, nutrient and gas exchange between the mother and fetus, and the removal of waste products from the fetus. In particular, vitamin A is transferred across the placenta via facilitated diffusion (Brown, 2008). Fetal growth and development are dependent on environmental factors such as energy, nutrients, and oxygen availability. As nutrient intakes fall below the recommended amounts, fetal growth and development are more compromised than maternal health. Vitamin A deficiency during pregnancy can cause malformations of the fetus' lungs, urinary tract, and heart (Brown, 2008).

Age-related macular degeneration occurs mostly after age 65, affecting the amount of lutein and zea-xanthin in the macula that protects tissue in the eye from blue light, which has photo-oxidative properties causing blurred vision. A more common result of VAD is the development of a cataract. A cataract is the partial or complete obstruction of the passage of light through the crystalline lens because of clouding. There are three different classes of xeropthalmia (childhood blinding disease caused by VAD): nyctalopia, keratomalacia, and Bidot's spots. Nyctalopia refers to night blindness, kertomalacia refers to the drying of the cornea, and Bidot's spots are corneal ulcerations where a foamy/cheesy material forms on the conjunctiva of the eye (Gropper *et al*., 2009). Corneal xerosis refers to the actual drying of the cornea, whereas keratomalacia is when the dryness turns to softening of the cornea. Bulging or rupturing of the cornea may ensue afterwards.

Vitamin A can be toxic when consumed at levels above the recommendation. Acute hypervitaminosis A results in nausea, vomiting, double vision, headache,

dizziness, and skin alteration. Follicular hyperkeratosis resembles goosebumps on the body, however when the skin is rubbed, the bumps do not disappear. Although carotene is non-toxic in excess levels (hypercarotenemia), it can cause the discoloration of the skin to an orange color (Gropper *et al*., 2009).

It was estimated that approximately 60 countries displayed the problem of VAD, with another 13 countries likely to face this problem in the future (World Health Organization, 2009). In the presence of VAD, children are also faced with various side affects ranging from several forms of vision loss to compromised immune function or an increased susceptibility to illnesses. Figure 2.5 depicts the areas most affected by vitamin A deficiency as determined by a serum retinol level of less than 0.70µmol/l.

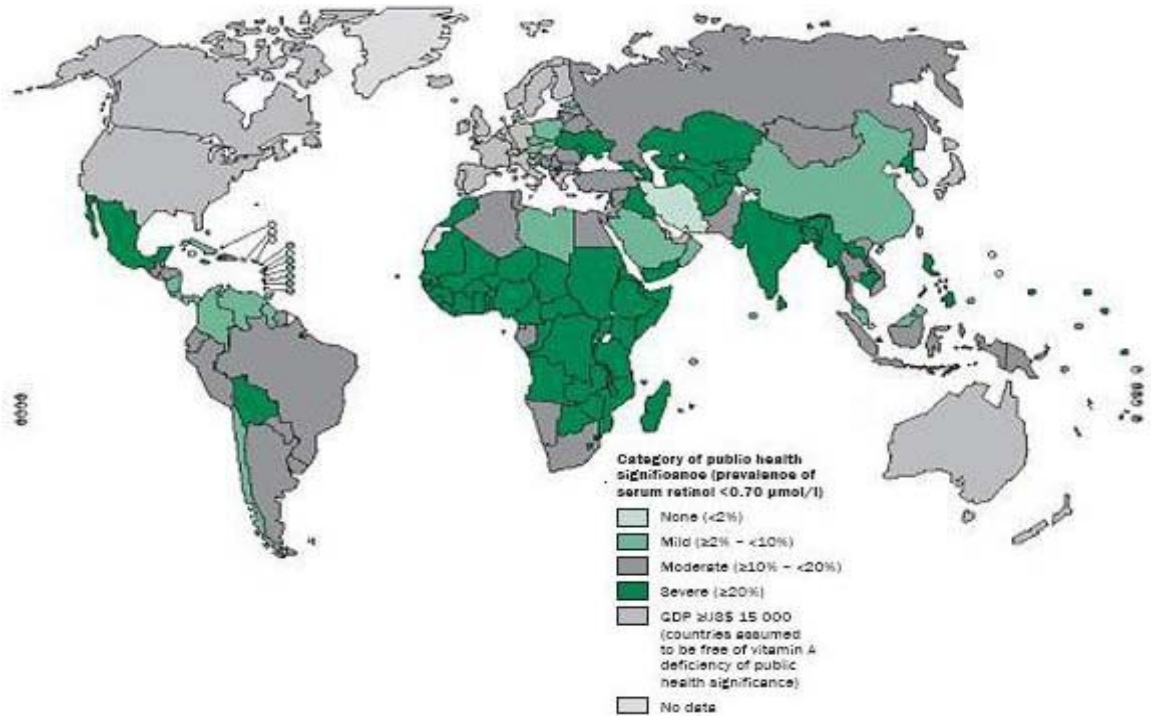

Figure 2.5 Category of Public Health Significance (Prevalence of Serum Retinol less than 0.70µmol/l). Source: Childinfo.org, 2009.

An example of how prevalent VAD is in developing countries, a study was conducted in India between 2002-2005 that specifically examined the effect of VAD and the effectiveness of vitamin A supplementation (VAS). The study discussed two significant points: (1) VAD is the most preventable cause of childhood blindness, and the resulting symptoms will eventually lead to total blindness and (2) the demise of the immune systems of children suffering from VAD are compromised leading to increased susceptibility of diarrhea, measles, and ultimately childhood death (Laxmaiah *et al.*, 2011). Laxmaiah *et al.* (2011) also reported that 40% of children's immune systems were comprised in developing countries due to VAD. Approximately 60,000 new cases of blindness are reported annually due to VAD in India. In 1970, the Government of India began the "massive-dose vitamin A supplementation program," which administers two doses (30,000µg and 60,000µg) of vitamin A with other vaccinations/booster shots biannually. This program was sponsored by the Ministry of Health and Family Welfare of the Indian Government. The recipients were infants and pre-school aged children of six months to five years of age. Even with the existence of the program for more than three decades, several areas of India are still suffering from childhood VAD. One result of the program is that there has been a decline in severe VAD manifested in the form of corneal ulcerations or keratomalacia (Laxmaiah *et al.*, 2011).

The National Nutrition Monitoring Bureau (NNMB) conducted a community-based cross-sectional study with a random sampling procedure in eight different states in India (Kerala, Tamil Nadu, Karnataka, Andhra Padesh, Maharashtra, Madhya Pradesh, Orissa, and West Bengal). These states were further broken down into villages and socioeconomic status. A total of 71,591 pre-school aged children from 48,232

households, and 2,681 mothers of the children, were examined for socioeconomic and demographic characteristics including community type, literacy status and occupation of parents, and size of family; knowledge and practices, and serum retinol levels. Also, collection of dried blood spots and clinical examination for ocular signs was conducted (Laxmaiah *et al.*, 2011). In reference to community type, the Indian Government has different classifications for its citizens based on their social or economic status: scheduled caste (SC), scheduled tribe (ST) and other backward classes (OBC). The SC and ST are exclusively known as the "underprivileged sections of society."

Information was obtained from the mothers in regards to the child(ren)'s history of night blindness. Questions were also asked regarding if their children experienced any hardship playing or identifying objects in dim light such as during sunset. A pre-tested, validated questionnaire was also administered to the mothers regarding their knowledge and practices of signs/symptoms, causes and consequences of VAD, and the use of VAS (Laxmaiah *et al.*, 2011).

Collection of blood spots were taken on a special chromatography filter paper. A finger puncture provided free-flowing blood in which two drops of blood were placed in an encircled area on the filter paper from each participant. The blood spots were left to air dry for one hour and then covered in black paper, put into an envelope with desiccant (a drying agent) and sent to the central reference laboratory (CRL) of NNMB every third day. Once at the CRL, the samples were frozen at -20ºC until analysis. Samples were kept in storage a maximum of six months before analysis, retaining retinol levels similar to initial levels. A team comprised of medical officers, nutritionists, and social workers were assigned to each state. At the CRL of NNMB, they were trained for three weeks

prior to testing in the field on how to identify signs/symptoms of VAD, collecting data on the nutrition knowledge and practices of mothers and collecting dried blood spots, packing, storing, and transporting of samples (Laxmaiah *et al.*, 2011).

It was revealed that 51.2% of the pre-school aged participants were boys. Forty-two percent of the children were from the OBC community, 28.2% belonged to forward castes, 19.4% were from SC, and ST represented 10.4%. Madhya Pradesh (40.8%) and West Bengal (40.3%) contained the most SC/ST populations. Occupation was highest in labor (45.3%), agriculture (27%) and service/business (27%). Half of the households contained two to four family members. Fifty-two percent of mothers were categorized as illiterate, comprising of 76.4% in Madhya Pradesh and 71% in Karnataka. Orissa (7.7%) and Madhya Pradesh (8.6%) had the lowest prevalence of sanitary toiletry access with Kerala having the highest. Maharashtra (1.1%) and Madhya Pradesh (0.8%) had the highest occurrence of night blindness. Madhya Pradesh (4.9%), West Bengal (3.7%) and Karnataka (2.2%) had the highest percentages for conjunctival xerosis (Laxmaiah *et al.*, 2011).

The prevalence of Bidot's spots was higher than 0.5% in six out of eight states, the highest being Madhya Pradesh (1.4%). The average signs/symptoms of VAD were a total of 2.3%, with the highest (6.6%) in Madhya Pradesh. Boys accounted for 2.6% of ocular VAD compared to girls (1.9%). Low serum retinol is defined as less than 20µg/dL. The mean serum retinol concentration of all child participants was 18.3µg/dL, with no differences between males and females. Although, serum retinol was significantly lower in children older than four years (17.5µg/dL) compared to younger children (19.0µg/dL) (Laxmaiah *et al.*, 2011).

Sixty-two percent was the overall occurrence of sub-clinical VAD in pre-school aged children, with children aged four years old representing 65.6% in comparison to all other ages (58-60%). There were different variations in each state, with Madhya Pradesh being the highest at 88% and the lowest in Tamil Nadu at 49%. The ST and SC communities had the highest rates (1.2-1.4%) of Bidot's spots compared to the other communities (0.4-0.6%). Bidot's spots were also more common among children whose parents were laborers (1.0%), illiterate mothers (1.1%) and children who were members of large families (0.9%) (Laxmaiah *et al.*, 2011).

In regards to the questionnaire that the mothers completed, 41% knew about night blindness, 8% knew about total blindness, and 4% of Bidot's spots as a result of VAD. Twelve to fourteen percent reported knowledge that symptoms of VAD stemmed from inadequate dietary intake or deficiency of vitamin A. Ten percent acknowledged that the prevention of VAD came from dietary intakes of green leafy vegetables, yellow and orange colored fruits, and animal products. Fifty-eight percent reported that their child(ren) had received at least one dose of VAS during the previous year, while 25% of mothers reported their child(ren) had received both VAS. Approximately one-third of mothers reported they did not know about the VAS program, while half of them stated that the program was never offered to their child(ren) and/or the time and place was not convenient. Only 13% acknowledged they had some type of health and nutrition education of signs/symptoms (5.3%), consequences of VAD (4.4%), VAS program (6.5%), and fruit and vegetable consumption (9.7%). Half of the mothers knew the benefits of VAS for eye health (Laxmaiah *et al.*, 2011).

It should be noted that severe signs of VAD such as corneal ulcerations and keratomalacia have become a rarity in diagnoses. However, Bidot's spots, conjunctival xerosis, and night blindness occur frequently in developing countries. The RDA for retinol is 400µg/day for β-carotene in pre-school aged children, but according to a survey conducted by the NNMB, 88% of the children were not meeting 50% of the RDA for vitamin A. Even as staggering as these figures are for VAD prevalence in India, these percentages are still lower than reports in developing countries such as Ethiopia. Even though the VAS program was a much needed program to implement in order to jumpstart the supplementation movement, it will not solve the entire problem. Laxmaiah *et al.* (2011) recommended not only supplementation but dietary diversity and fortified staple food items to help battle the VAD crisis in developing countries.

Sweet Potatoes (*Ipomoea batatas*)

Believed to be the least expensive plant source of β-carotene, orange-fleshed sweet potatoes are an important source of dietary vitamin A to poor rural families (Eluagu and Onimawo, 2010). Sweet potatoes can be grown on marginal and degraded soil and therefore are utilized as a widely grown cash crop. Orange-fleshed sweet potatoes are implemented in food diversification programs in Sub-Saharan Africa to help counteract vitamin A deficiency (Mahlangeni *et al.*, 2012). There are varying colors of sweet potatoes, ranging from white, yellow, orange, and purple. However, the most cultivated and familiar one is the orange-fleshed sweet potato.

Sweet potatoes are good sources of β-carotene, dietary fiber, and minerals (Leksrisompong *et al.*, 2012). As a pro-vitamin A compound, β-carotene is a precursor to

vitamin A and prevalent in orange-fleshed sweet potatoes. According to the United States Department of Agriculture (n.d.), one 5-inch long, raw sweet potato weighing 130g contains 112 kilocalories, 26.16g total carbohydrates, 2.04g protein, 0.06g total fat, 18,443 IU of vitamin A, 11,062µg beta-carotene, 3.1mg vitamin C, 0.272mg vitamin B6, 3.9g total dietary fiber, 438mg potassium, 0.335mg manganese, and 72mg of sodium.

Vitamin C, also known as ascorbic acid, functions as an antioxidant in plant cells. Vitamin c has been associated with protecting against heart disease, high cholesterol, high blood pressure, and reduced gastric cancer risk. The β-carotene present in sweet potatoes also acts as an antioxidant, possessing cancer-preventative activities and reduced occurrences of cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to low immune function, and other degenerative diseases. Additionally, sweet potatoes also contain phenolic compounds which serve as anti-oxidative compounds. Their presence has been associated with reduced occurrences of melanogenesis, hepatoma invasion, and HIV replication (Antonious *et al.*, 2011).

Sweet potatoes contain relatively high amounts of phenolic compounds. Chlorogenic acid, caffeic acid, and isomers of dicaffeoylquinic acid serve as the main phenolic acids in sweet potato cultivators, harboring anti-mutagenic and anti-oxidative properties in combating free radicals. Polyphenolic compounds assist in reduced risk of cancers, neurodegenerative diseases, and prevent oxidation of biomolecules such as DNA, lipids, and proteins. Polyphenols and tannins may also play a role in preventing type 2 diabetes by affecting glucose absorption and related hormones. Carotenoids and phenolic compounds, other than being known for their anti-oxidative properties, also give

this starchy vegetable its flesh color, which ranges from cream, deep yellow, orange, to purple (Antonious *et al.*, 2011).

Based on a consumer acceptance study that was conducted to examine the various flesh colors, textures, and flavors of sweet potatoes, descriptors for the orange-fleshed sweet potatoes included fibrous, moist, residual fiber texture, earthy/canned carrot, dried apricot/floral flavor and aroma, visually moist, high color homogeneity, and sour taste. Consumption of orange-fleshed sweet potatoes in the United States is very minute, accounting for only 2.3kg per capita. With sweet potatoes being a staple food in developing countries, some declare it the sixth most important crop worldwide, preceded by wheat, rice, maize, potato, and cassava (Leksrisompong *et al.*, 2012). Others proclaim it the seventh most important crop with the inclusion of barley (Antonious *et al.*, 2011).

The Pacific Islands and Asia account for the largest production of sweet potatoes, representing 93% of global distribution. China is third, accounting for 80% of sweet potato production globally. The United States is among the lowest sweet potato production harboring only 0.8% of global sweet potato production (Leksrisompong *et al.*, 2012). Overall, sweet potatoes have had a world production of about 130 million tons, ranking third most important starchy root vegetable preceded by cassava and potatoes (Antonious *et al.*, 2011). In addition to sweet potatoes being used as a starchy vegetable, researchers are interested in turning this vegetable into a functional ingredient produced in various forms such as purees or flour (Leksrisompong *et al.*, 2012).

Sensory Analysis

Sensory analysis is the measurement and analysis of human responses to the characteristics of foods and beverages regarding their appearance, feel, odor, texture,

temperature and taste (Anonymous, 2010; Meilgaard *et al*., 2007). Typically, the most frequently used scale for determining the acceptability of a food product is the 9-point hedonic scale that ranges from 'like extremely' to 'dislike extremely' (Peryam and Pilgrim, 1957; The Society of Sensory Professionals, 2010). However, various other hedonic scales referred to as "pictorial hedonic scales" have been formulated, containing words, pictures (smiley faces) or a combination of both that are geared specifically towards young children (Popper and Kroll, n.d.). The terms "like extremely" and "dislike extremely" are omitted for more child-friendly terms such as "super-good" or "super-bad" to describe their preference for the food product (Popper and Kroll, n.d.). In addition to determining the subjective acceptability of foods through sensory analysis, other objective analyses can determine nutrient contents of food.

High-Performance Liquid Chromatography

"High Performance Liquid Chromatography (HPLC) is a rapid, efficient and sensitive technique for carotenoid analysis" (Ahamad *et al*., 2007). Carotenoids are very susceptible to oxidation in the presence of oxygen, light, heat, and other various oxidizing agents; therefore, extreme care must be taken when conducting carotenoid analysis. The identification of which carotenoid needs to be extracted determines the specific method that will be used in HPLC, keeping in mind that not all carotenoids possess significant vitamin A activity. All compounds of interest need to be extracted from the product, most commonly using the solvents acetone, hexane or tetrahydrofuran to remove the color (β-carotene) (Aalbersberg, n.d.). Shahid (2007) defines chromatography as the following:

> It is the separation technique in which the mixture to be separated is in mobile phase which is made to move in contact with a selectively absorbent stationary

phase. It can be an analytical method, examining the number and nature of the components in a very small amount of a mixture, but does not actually isolate them. Or it can be a preparative method, which investigates a large quantity of the mixture to obtain useable amounts of each component.

According to The Linde Group (2008), HPLC is a type of column chromatography test that uses high pressure (typically in the form of nitrogen) to push the sample mixture or substance of interest through a column containing chromatographic material. There are two different phases to HPLC: mobile phase and stationary phase. Columns used in HPLC vary in size (length and internal diameter) and polarity. For example, polar silica is placed inside the column, having a non-polar solvent such as hexane. When the solvent or substance is passed through the tube through high pressure, the polar compounds will "stick" to the polar silica, allowing the non-polar compounds to quickly pass through. This is known as normal phase HPLC; however, the opposite can also be done (column filled with non-polar particles allowing polar compounds to pass through) which is known as reversed phase HPLC (Clark, 2007). The passing or pumping of the solvent at high pressure is the mobile phase. The sticking or passing of the solvent through the column is the stationary phase (The Linde Group, 2008) or the stationary bed (Shahid, 2007).

It is important to note that the sample being tested using HPLC must be soluble in the selected solvent for the mobile phase. However, if the sample is too soluble, it will cause the solvent to move too quickly through the stationary phase. This will result in a lack of separation of the components. All components have different rates or times in which they pass through the "medium," referred to as retention time (Shahid, 2007).

According to Aalbersberg (n.d.), a proposed retention time for β-carotene is 10.8 minutes. Proposed retention times of other carotenoids include: alpha-carotene (10.2 minutes), beta-cryptoxanthin (6.0 minutes), lutein (3.3 minutes) and zea-xanthin (3.3 minutes) (Aalbersberg, n.d.).

Determining the β-carotene content in a sweet potato product using the HPLC process will assist in determining the product's possible contribution to assisting in VAD. However, it is not only important to test the β-carotene content, but to also test the acceptability of the product. It was hypothesized that a cookie product containing sweet potato would be a good source of β-carotene and also be accepted among pre-school aged children and adults.

CHAPTER III

MATERIALS AND METHODS

Sweet Potato Cookie Preparation

The preparation of the cookie samples occurred in the James E. Garrison Sensory Evaluation Laboratory at Mississippi State University. Preliminary research determined that a cookie containing wheat flour, a cookie containing a gluten-free flour mix instead of wheat flour, and a cookie containing a gluten-free flour mix instead of wheat flour with extra sweet potato would be used for this research project. The ingredients of the gluten-free sweet potato cookies are listed by measurement and weight as modified from Cooks.com (Anonymous, 2013). All ingredients were obtained from Kroger® grocery store in Starkville, Mississippi. The cookies were prepared the day before analysis of β-carotene occurred; approximately 17 hours and 15 minutes prior to the time of analysis.

The oven was pre-heated to 350 degrees Fahrenheit. One medium-sized sweet potato was baked for approximately an hour on the convection oven setting. Butter, sugar, eggs, and vanilla extract were creamed together in a large mixing bowl. In another large mixing bowl, the salt, baking powder, baking soda, allspice, and flour were sifted together. The dry mixture was incrementally combined with the wet ingredients, mixing between additions until all of the dry ingredients were incorporated. The baked sweet potato and chopped dates were the final ingredients to be incorporated into the mixture.

Two cookie sheets were evenly sprayed with butter-flavored cooking spray (Kroger®, Cincinnati, OH) to prevent sticking. A spoonful of the cookie mixture was dropped onto the cookie sheet and pressed or semi-flattened with a spoon. The cookies were baked at 350 degrees Fahrenheit for approximately 18 minutes. Once they had cooled completely on metal racks, they were stored in a gallon-size zip-lock bag overnight in an enclosed, dark cabinet located in the Sensory Laboratory until used for analysis the next day.

Table 3.1 Gluten-Free Sweet Potato Cookies Recipe

Ingredients	Measurement	Weight (grams)
Butter, softened (Imperial®, Englewood Cliffs, NJ)	½ cup	113 grams
Light brown sugar (Kroger®, Cincinnati, OH)	1 cup	170 grams
Sweet potatoes, peeled, mashed	1 cup	190 grams
Gluten-free flour (Gluten-Free Pantry®, Paramus, NJ)	2 ½ cups	281 grams
Salt (Kroger®, Cincinnati, OH)	1 teaspoon	7 grams
Eggs, large, beaten (Kroger®, Cincinnati, OH)	2	100 grams (50g each)
Baking powder (Kroger®, Cincinnati, OH)	2 teaspoons	8 grams
Baking soda (Kroger®, Cincinnati, OH)	½ teaspoon	3 grams
Allspice, Ground Jamaican (McCormick®, Hunt Valley, MD)	½ teaspoon	1 grams
Vanilla flavor, Imitation (Kroger®, Cincinnati, OH)	1 teaspoon	5 grams
Dates, chopped (Sun-Maid®, Kingsburg, Ca)	½ cup	73 grams

Source: Anonymous, 2013

Development of Beta-Carotene Standard Curve

β-carotene standard curve development took place in the Mississippi State Chemical Laboratory on the Mississippi State University campus. β-carotene (51.7mg) obtained from Sigma-Aldrich (St. Louis, MO, USA) was weighed and dissolved in 10ml

of chloroform in a 100ml volumetric flask. Once the dissolution of β-carotene was complete, the solution was further diluted to the 100ml mark with methanol and inverted. Ten milliliters of this primary stock were then added to another 100ml volumetric flask. The remainder of the volume was made up by one part acetone to nine parts hexane (10ml acetone and 90ml hexane). A range of 300 to 600 nm was used to observe the absorbance of β-carotene by scanning the secondary stock solution through a Hewlett Packard 8453 UV-VIS spectrometer (Santa Clara, CA, USA) with a quartz curvette of 1cm path-length (Sullivan, 1993).

An amount (12.5µg/ml) of the primary β-carotene stock solution was added into a vial designed for a Waters 2690 Alliance High-Performance Liquid Chromatograph (Milford, MA, USA) with a 2487 Waters UV-VIS spectrometer (Milford, MA, USA). The column used was an Xterra C18 stationary phase having a dimension of 250x4.6mm with a guard column of C18 stationary phase (Milford, MA, USA). The mobile phase used was 90/10ml methanol/chloroform and was filtered with a 0.45µm disk filter with a vacuum. The flow rate was set at 1ml/min with a wavelength set at 475nm (Sullivan, 1993). The HPLC was run at 5, 10, 20, 30, and 40µl to obtain a standard curve (r^2= 0.9981).

Determination of Beta-Carotene Content

The HPLC method used for β-carotene analysis was from *Methods of Analysis for Nutrition Labeling* (Sullivan, 1993). β-carotene analysis took place in the Mississippi State Chemical Laboratory. On the day of analysis, the cookies were weighed in triplicate to approximately 10g each to represent three trial analyses of β-carotene content. Each of

the replicates conducted used cookie samples from the same batch. For each trial, the cookies were transferred to a blender with 100ml of one part acetone (10ml) to nine parts hexane (90ml) and blended for five minutes into a slurry. Under a stream of nitrogen gas, 5ml of the acetone/hexane solution was evaporated from the β-carotene residue. The β-carotene residue was dissolved in 2ml of a 90ml methanol and 10ml chloroform solution, sonicated, and vortexed. The β-carotene mixture was then added to a syringe with a terminal filter of .45µm pore size to ensure that no un-dissolved particles were present. The filtrate was collected into a vial (Sullivan, 1993).

A Waters 2690 Alliance High-Performance Liquid Chromatograph with a 2487 Waters UV-VIS spectrometer (Milford, MA, USA) at 325nm was used for β-carotene content analysis. An Xterra C18 stationary phase with a dimension of 250x4.6mm with a guard column of C18 stationary phase (Milford, MA, USA) was used as well. Each of the three trials had a run time of ten minutes, an injection volume of 20.0µl, and a flow rate of 1ml/min. Final concentrations of β-carotene in the samples were calculated using the standard curve (Sullivan, 1993).

Sensory Evaluation

The experiment conducted for this section was to evaluate consumers' preference or non-preference for sweet potato cookie products with respect to appearance, aroma, flavor, texture, and overall acceptability. It was important to not only analyze adults' reaction to the product, but pre-school aged children as well given that the product was meant to ultimately benefit young children.

Sensory Evaluation by Adults

All three sensory evaluations and the baking of the sweet potato cookie products occurred in the James E. Garrison Sensory Evaluation Laboratory. The cookies were prepared one day before sensory panels occurred. The participants of this study were recruited from Mississippi State University via an approved email. The three sensory panels occurred on three different days between the hours of 10 a.m. and 12:30 p.m.

Adults were asked to analyze and rate three different sweet potato cookie products; the first was sweet potato cookies containing wheat, the second was the modified recipe of gluten-free sweet potato cookies, and the third was a modified recipe of gluten-free sweet potato cookies with extra sweet potato added. All sweet potato cookie products were derived from the same recipe described in Table 3.1, with the second and third samples having slight modifications. The only difference in the recipe of the cookie containing wheat was that it contained wheat flour. Instead of substituting for gluten-free flour as was done for the second and third samples, all-purpose flour was used for the cookie containing wheat. The recipe for the gluten-free sweet potato (GFSP) cookie samples were the same as the gluten-free sweet potato cookies, except the GFSP sample had approximately 48 grams of additional cooked sweet potato added to it. The same baking techniques as described above were utilized in the preparation of these samples.

On the morning of the sensory panels, each of the whole cookie samples were cut into small rectangles, approximately six pieces per cookie, and cupped into three small, separate plastic containers with tops (Dart®, Mason, MI). Each of the three containers had a random three-digit number on them to represent the samples. A white and yellow

overhead light was used to light the tasting booths in the James E. Garrison Sensory Evaluation Laboratory. Participants were provided cookie samples, a cup of water, an expectorant cup, a napkin, and a pencil. A score sheet was also provided for the participants to evaluate each individual sample on appearance, aroma, flavor, texture, and overall acceptability.

A 9-point hedonic scale ranging from 1= dislike extremely to 9= like extremely was used to analyze the participants' preference of sweet potato cookie products (Appendix A) (Peryam and Pilgrim, 1957; The Society of Sensory Professionals, 2010). Participants were also asked to rank the samples from one to three, one representing the 'best liked' and three representing the 'least liked.' No personal information was required by participants other than their gender and age group. Other information was collected in reference to their prior history of consumption of sweet potatoes and gluten-free products.

Sensory Evaluation by Pre-School Aged Children

The baking of the sweet potato cookie products and sensory evaluation occurred in the Child Development and Family Studies Center that is affiliated with Mississippi State University. The cookies were prepared one day before sensory panels occurred. The participants were recruited via a parental consent form that was administered to the parents of the children through the child center staff. The sensory panel took place between the hours of 9 a.m. to approximately 11 a.m.

Pre-school aged children were asked to analyze and rate two different sweet potato cookie products; the sweet potato cookies containing wheat and the gluten-free sweet potato cookies. Both sweet potato cookie products were produced from the same

recipes as the samples for adults. On the morning of the sensory panel, each of the whole cookie samples were cut into small rectangles, approximately six pieces per cookie, and cupped into two small, separate plastic containers with tops (Dart®, Mason, MI). Each of the two containers had a random three-digit number on them to represent the samples. The children were escorted into an unused room in the child center, individually, by a teacher employed by the center who volunteered to assist. Participants were provided cookie samples, a cup of water, an expectorant cup, and a napkin. A 3-point smiley face hedonic scale consisting of 1= dislike, 2= neither like or dislike, and 3= like was used to analyze the children's preference of the sweet potato cookie products (Appendix A) (Popper and Kroll, n.d.). No personal information was required by participants other than their gender and age. This information was gathered solely for the purpose of statistical analysis.

Institutional Review Board Approval

Approval from the Mississippi State University Institutional Review Board (IRB Study #13-206) for Research with Human Subjects was obtained prior to beginning the study (Appendix B). All adult participants of the consumer sensory panel for sweet potato cookies provided written, informed consent before evaluating any samples. In compliance with IRB regulations, a list of all ingredients of the samples was provided to participants before the sensory panels were conducted to prevent any potential allergic reactions.

Approval for the Mississippi State University Institutional Review Board (IRB Study #13-218) for Research with Human Subjects who were minors (under 18 years of age) was obtained before consumer panels occurred at the Child Development and Family Studies Center (Appendix B). All parents or guardians of participants for the

consumer sensory panel for sweet potato cookies provided written, informed consent before any samples were evaluated (Appendix B). In compliance with IRB regulations, a list of all ingredients of the samples was provided to all parents, guardians, and participants before the sensory panels were conducted to prevent any potential allergic reactions.

Statistical Analysis

Using Statistical Analysis Software (SAS v. 9.3, SAS Inst. Inc., Cary, North Carolina, USA), a randomized complete block design with three replications (n= 148) was utilized in order to determine consumer acceptability ($p<0.05$) of the sweet potato cookies. The Least Significant Difference (LSD) was used to distinguish treatment means in the occurrence of significant differences between treatments. Participants were further clustered together in five separate groups using dendrogram and dissimilarity plots, based on their acceptance of sweet potato cookies by agglomerative hierarchical clustering. Randomized complete block designs were used to distinguish ($p<0.05$) between sweet potato cookies within each cluster. In the occurrence of significant differences, the LSD test was used to separate treatment means (Meilgaard *et al*., 2007; Schilling and Coggins, 2007).

CHAPTER IV

RESULTS AND DISCUSSION

Beta-Carotene Content

Three replications were conducted to determine the β-carotene content of gluten-free sweet potato cookies using HPLC. Only the gluten-free sweet potato cookies were used for β-carotene analysis because this was the main product of interest to possibly be introduced to the marketplace. Also, during preliminary testing of the recipe, it was especially accepted by peers. Based on the results from the three replications, it was determined that the samples contained 11.8 parts per million (ppm), 8.7 ppm, and 9.7 ppm of β-carotene. After averaging these values, it was concluded that the gluten-free sweet potato cookies contained approximately 10.1 ppm, or 10.1 µg of β-carotene per gram of cookie. As referenced in Table 1.1, 12 µg of β-carotene is equivalent to 1 µg of retinol (vitamin A). Therefore, we can calculate that a 20-gram gluten-free sweet potato cookie contained 202 µg of β-carotene per cookie, which would be equivalent to 16.8 µg of vitamin A (retinol).

Sensory Evaluation

Adults

The majority (63.9%, n=99) of participants were women, 32.9% were men (n=51), and 3.2% (n=5) did not provide a response. Most (81.3%, n=126) of the

participants were 18 to 29 years old, 5.8% (n=9) were 30 to 39 years old, 5.2% (n=8) were 40 to 49 years old, 6.5% (n=10) were 50 years and older, and two participants did not indicate their age group. Participants were asked, "In general, do you like sweet potatoes?" Fifty percent (n=78) of the respondents reported they very much liked sweet potatoes, 37.4% (n=58) indicated they somewhat liked sweet potatoes, 7.7% (n=12) reported they neither liked nor disliked sweet potatoes, and 3.2% (n=5) indicated they somewhat disliked sweet potatoes. No one reported they very much disliked sweet potatoes. Participants were also asked, "How often do you usually eat sweet potatoes?" Responses ranged from never (3.2%, n=5) to eating sweet potato products three or more times a week (5.8%, n=9) with 52.2% (n=81) participants stating they consumed sweet potato products one to three times a month to one time a week. As expected, there was a positive correlation between participants who stated they liked sweet potatoes and how often they consumed sweet potato products ($r=0.399$, $p<0.001$).

Regarding the appearance of the three different samples of sweet potato cookies, there were no significant differences. The wheat and the GFSP treatments were both rated with a mean score of 7.3 and the gluten-free treatment was rated similarly with a score of 7.1 (Table 4.1). In the case of aroma, there was a preference ($p<0.05$) for the wheat sample over the GFSP. The flavor of the wheat (7.0 mean score) and GFSP treatments (6.7 mean score) was preferred ($p<0.05$) over the gluten-free treatment (6.2 mean score). There was a significant difference ($p<0.05$) between the wheat and GFSP samples when compared to the gluten-free sample in reference to texture. The gluten-free sample had a mean score of 6.2 compared to a mean of 6.8 ($p<0.05$) for both wheat and GFSP samples. Analysis of the overall acceptability of the treatments determined that the wheat and

GFSP treatments were preferred over the gluten-free treatment ($p<0.05$). As summarized in Table 4.1, the affinity of panelists for sweet potato cookies was observed to be for the wheat and GFSP samples. Although the GFSP sample was not significantly different ($p>0.05$) from the wheat sample for overall acceptability, the gluten-free sample was significantly different ($p<0.05$) and was liked less (Table 4.1).

Table 4.1 Mean Scores for Consumer Acceptability (n=148) of Three Sweet Potato Cookie Samples[1]

Cookie Samples	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Wheat	7.3[a]	7.4[a]	7.0[a]	6.8[a]	7.1[a]
Gluten-Free	7.1[a]	7.2[ab]	6.2[b]	6.2[b]	6.2[b]
Gluten-Free with Extra Sweet Potato (GFSP)	7.3[a]	7.0[b]	6.7[a]	6.8[a]	6.8[a]

[ab]Mean scores within a column with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point hedonic scale (1= dislike extremely to 9= like extremely)

It is important to note that there was a difference between the number of participants analyzed in the different categories of acceptability (n=148) and the participants that were clustered into five separate groups (n=144). The removal of four incomplete surveys prior to cluster analysis accounted for this difference. Eighteen percent of participants (cluster one) had a preference ($p<0.05$) for the GFSP cookie compared to the gluten-free and wheat cookies. Forty-four percent of participants (cluster two) preferred ($p<0.05$) the sweet potato cookie containing wheat as opposed to the GFSP and gluten-free cookies. Seventeen percent of the participants (cluster three) preferred ($p<0.05$) the GFSP and gluten-free samples rather than the wheat cookie

sample. Cluster four (10% of participants) had a preference ($p<0.05$) for the wheat (mean score of 7.1) and gluten-free cookies (6.5 score) compared to the GFSP cookies (mean score of 4.0). Thirteen percent of participants (cluster five) had a high affinity ($p<0.05$) for the GFSP (7.1 score) and wheat (6.7 score) cookies over the gluten-free (3.6 score) cookies. Table 4.2 provides a summary of these results.

Table 4.2 Mean Scores for Overall Consumer Acceptability of Sweet Potato Cookie Samples According to Different Clusters of Consumer Segments Using a Hedonic Scale[1]

Cluster	Consumers (n)	Percentage (%)	Wheat	Gluten-Free	Gluten-Free with Extra Sweet Potato (GFSP)
1	26	18	5.0^{b}	4.6^{b}	5.9^{a}
2	63	44	7.5^{a}	6.8^{b}	6.9^{b}
3	24	17	8.2^{b}	8.3^{ab}	8.6^{a}
4	13	10	7.1^{a}	6.5^{a}	4.0^{b}
5	18	13	6.7^{a}	3.6^{b}	7.1^{a}

[ab]Mean scores within a row with different letters are significantly different ($p<0.05$)
[1]Scores were based on a 9-point hedonic scale (1= dislike extremely to 9= like extremely)

Given the fact that these sweet potato cookie products may possibly provide pre-school aged children with a source of vitamin A, one may wonder why it was decided to conduct sensory analysis with adults, as well as children. Because adults or parents would actually be the consumer purchasing this product, it was important to ensure that their approval for the product was recognized. Parents tend to base their children's dietary intakes from their own. Therefore, it was crucial to ensure that adults rated the products

as acceptable, which they did according to Table 4.1. Furthermore, cluster three (17% of adult participants) rated the sweet potato cookies very high with scores of 8.2, 8.3, and 8.6 using a 9-point scale.

Pre-School Aged Children

There were eight girls and seven boys with parental/guardian informed, written consent that participated in the sensory study at the Child Development and Family Studies Center. Three year olds was the age group most represented (n=10), followed by four year olds (n=5). The majority of participants were Caucasian (n=11), followed by Asian (n=2), African American (n=1) and Grecian (n=1). Since there was a limited number (n=15) of pre-school aged children that participated in the sensory evaluation; only a randomized complete block design with one replication was used to differentiate between the consumer acceptability of two sweet potato cookie products. The LSD was used to distinguish between treatment means in the occurrence of significant differences between treatments (Meilgaard *et al*., 2007; Schilling and Coggins, 2007). One child tasted the wheat-containing sweet potato cookie but refused to taste the gluten-free cookie. There was no significant difference ($p>0.05$) between children's preference for the gluten-free sample and the wheat sample. Overall, the children liked both samples and mean scores were 2.7 for the sweet potato cookie containing wheat and 2.9 for the gluten-free sweet potato cookie (Table 4.3).

Table 4.3 Mean Scores for Children's Overall Acceptability of Two Sweet Potato Cookie Samples[1]

Sweet Potato Cookie Samples	Children (n)	Overall Acceptability
Wheat	15	2.7[a]
Gluten-Free	14	2.9[a]

[ab]Mean scores within a column with the same letter are not significantly different ($p>0.05$)
[1]Scores were based on a 3-point hedonic scale (1= dislike, 2= neither like or dislike, and 3= like)

Limitations

One limitation of this study was the participants' perception of what a 'cookie' should be. The recipe that was modified from *Cooks.com*, referred to the end products as being cookies. However, when the products were baked and cooled, they had the appearance and texture of muffin tops. Reviewing the data and comments from the adult hedonic score sheets, several comments were made referring to the samples as being "Too thick" or "Too cake-like" to be cookies.

Several preliminary tests were conducted with the recipe prior to the start of this research without any modification to the recipe, and those products were similar to the products used in this study. This may have had a somewhat negative affect on the participants liking or disliking the product simply based on their perception of a cookie's characteristics. Many comments praised the aroma, appearance, and taste of the cookies, but stated that the texture did not match that of a cookie.

Working with pre-school aged children was also a limitation. There were more three year olds participating than four and five year olds, and sometimes it was difficult

keeping their attention on the task at hand or cooperating at all. It is possible that the thrill of being singled out to leave the classroom while the other children had to stay, and the fact that they would be tasting cookies was enough for them to be happy and 'like' the product when asked by the interviewer. However, it was observed that the majority of the children seemed to genuinely like the products. Working with older children or more four and five year olds may have provided more accurate results of whether they truly accepted the cookies than with the three year olds. Although, conducting sensory analysis research with the children was beneficial. Due to the small sample size of children, the sensory analysis results cannot be generalized to other children that did not participate in this study.

CHAPTER V

CONCLUSION

The WHO indicated that vitamin A deficiency remains a significant public health problem at a global level. This is attributed to high rates of death in children under the age of five years (World Health Organization, 2009). The recommended dietary allowance for vitamin A in children ages one to eight years is 300-400µg/day (Institute of Medicine, Food and Nutrition Board, 2004). The WHO recognizes three different deficiency control strategies: supplementation, food fortification, and dietary diversity (World Health Organization, 2009). Through the exploration of food sources that contain vitamin A, and vitamin A precursors such as β-carotene, the possible utilization of sweet potatoes in the unique form of a cookie may improve the intake of vitamin A in children. This study determined the β-carotene content of gluten-free sweet potato cookies and determined consumer acceptability of sweet potato cookie products among adults and pre-school aged children.

Vitamin A is a fat-soluble vitamin, whose primary function is providing visual ability through the cleaving of rhodopsin in the retina of the eye (Gropper *et al*., 2009). Vitamin A is an umbrella term for the biological activity of retinol (Helwig, n.d.). Retinol is recognized as preformed vitamin A, signifying that it is the primary form of vitamin A

utilized in the body. In order for optimal absorption of vitamin A, there must be 10g or more of fat present (Gropper *et al*., 2009).

Carotenoids are precursors to vitamin A, also known as provitamin A compounds, with the most common and potent being β-carotene. After the consumption of β-carotene, it is converted to the retinol form to be used in the body (Gropper *et al*., 2009). Twelve micrograms of dietary β-carotene equals one microgram of retinol (Drake *et al*., 2007). Retinol cannot be produced in animals or humans and must be obtained through dietary intake (Gropper *et al*., 2009). Orange-fleshed sweet potatoes are an excellent source of dietary β-carotene. According to the United States Department of Agriculture (n.d.), one 5-inch raw sweet potato weighing 130g contains 11,062µg of β-carotene.

The HPLC method was used to investigate the β-carotene content of gluten-free sweet potato cookies, resulting in approximately 10.1 ppm, or 10.1µg of β-carotene per gram of cookie. Also, sensory evaluation was conducted among adults and pre-school aged children to evaluate preference for the sweet potato cookies. The three samples evaluated were gluten-free, gluten-free with extra sweet potato (GFSP), and wheat-containing sweet potato cookies. They were evaluated for appearance, aroma, flavor, texture, and overall acceptability. A 9-point hedonic scale ranging from 1= dislike extremely to 9= like extremely was used for adults. A 3-point hedonic scale consisting of 1= dislike, 2= neither like nor dislike, and 3= like, was used for the children.

Results for the adults concluded that participants' rated the overall acceptability of the wheat-containing and GFSP cookies higher ($p<0.05$) than the gluten-free cookies. There were no significant differences ($p<0.05$) among the three samples for appearance. The texture of the wheat and GFSP cookies was scored higher than the texture for the

gluten-free cookie. Flavor and overall acceptability were rated similar between the wheat and GFSP samples, both higher than the gluten-free sample rating. Three out of the five clusters had a higher affinity ($p<0.05$) for GFSP and wheat-containing samples, while only two of the five clusters had a higher affinity for the gluten-free samples. Regarding the results for the pre-school aged children, they liked both the gluten-free cookie and the wheat-containing sweet potato cookie.

The data collected from this research may justify the use of not only sweet potatoes, but the use of sweet potato cookies in children and adults to combat vitamin A deficiency. Although these products were not a significant source of β-carotene or vitamin A, they could be used as a supplemental product in addition to the supplemental practices already in place for VAD. Other alternative sweet potato products could be explored as well, such as the use of sweet potato flour to make various food items. A study conducted by Bechoff *et al*. (2011), explored the use of orange-fleshed sweet potato flour in blended foods in Uganda. Foods were prepared such as porridge, mandazis (square and spongy cakes) and chapatis (large pancakes) using different ratios of orange-fleshed sweet potato flour and other types of flour (Bechoff *et al*., 2011). Even if not in the form of a cookie, there are several ways that sweet potatoes could be utilized other than in the regular tuber form. With additional creative culinary experiments and analysis on how specific preparation/cooking methods affect the stability of β-carotene content of the sweet potato, multiple products containing vitamin A could be developed to reach more children in need of VAD relief.

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APPENDIX A

SCORE SHEETS FOR CONSUMER SENSORY PANELS

Evaluation of Sweet Potato Products **Date:**

1. What is your gender? ___Female ___Male

2. What is your age group? ___18–29 ___30–39 ___40–49 ___50 years or older

3. In general, do you like sweet potatoes?

____Yes, like very much
____Yes, like somewhat
____Neither like nor dislike
____No, dislike somewhat
____No, dislike very much

4. How often do you usually eat sweet potato products?

____3 or more times a week
____2 times a week
____1 time a week
____Less than once a week
____One to three times a month
____Less than once a month but a few times a year
____Never

5. Have you eaten gluten-free sweet potato cookies? ___Yes ___No

6. Do you eat gluten-free products? ___Yes ___No

7. If you answered "yes" above, why do you eat gluten-free products?

For the cookie samples, **please evaluate each sample independently**. After tasting the food product, if you do not wish to swallow the sample, you may expectorate in the cup and rinse with the water provided.

Each column will need one check mark if you choose to evaluate all samples.

Sample 586	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Please continue on the other side →

Sample 109	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Sample 352	Appearance	Aroma	Flavor	Texture	Overall Acceptability
Like extremely					
Like very much					
Like moderately					
Like slightly					
Neither like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

Please rank the two samples from 1 to 2 with **1 being best liked** and 3 being least liked:

Cookie Samples	Your Ranking
586	
109	
352	

Please feel free to provide any comments _____

_____

_____

Thank you for your participation!

MSU IRB #13-206
Approved: 07/19/13
Expires: 07/15/18

Consumer Acceptance of Sweet Potato Cookies in Pre-School Aged Children Script

Interviewer: Hello. My name is _____ and I need a favor. I am going to ask you to taste two different cookies, and point to the smiley face that best describes how you feel about the cookie. Is that ok? Do you understand or have any questions?

Subject: Yes or no…

Interviewer: Here is cookie number one (gives child cookie sample).

Subject: (Child tries cookie. He or she may spit the sample out in an empty paper cup if they choose not to swallow).

Interviewer: (Places survey sheet in front of child) Please point to the smiley face that best describes the taste of the cookie.

Subject: (Child points to picture)

Interviewer: Thank you. Would you like a drink of water?

Subject: Yes or no…

Interviewer: Here is cookie number two (gives child cookie sample).

Subject: (Child tries cookie. He or she may spit the sample out in an empty paper cup if they choose not to swallow).

Interviewer: (Places survey sheet in front of child) Please point to the smiley face that best describes the taste of the cookie.

Subject: (Child points to picture).

Interviewer: Thank you. Would you like a drink of water?

Subject: Yes or no…

Interviewer: Thank you for your help!

Evaluation of Sweet Potato Cookies by Pre-School Age Children

Directions: Each child will taste a cookie sample and be asked to point to the face that best describes how he or she likes or dislikes the sample. A drink of water will be offered in between sample tasting.

Boy or Girl _____ years old Date: _____

Sample name	1. Dislike 	2. Neither like or dislike 	3. Like 	Comments
Gluten-free sweet potato cookie				
Sweet potato cookie (Contains wheat)				

APPENDIX B

INFORMED CONSENT AND MISSISSIPPI STATE UNIVERSITY INSTITUTIONAL REVIEW BOARD APPROVAL

Diane Tidwell - Study 13-206: Consumer acceptance of sweet potato cookies

From:
To:
Date: 7/19/2013 1:43 PM
Subject: Study 13-206: Consumer acceptance of sweet potato cookies
CC: , ,

July 19, 2013

Aja Stokes
Dept. of Food Science, Nutrition and Health Promotion
Mississippi State University

RE: HRPP Study #13-206: Consumer acceptance of sweet potato cookies

Dear Ms. Stokes:

This email serves as official documentation that the above referenced project was reviewed and approved via administrative review on 7/19/2013 in accordance with 45 CFR 46.101(b)(6). Continuing review is not necessary for this project. However, in accordance with SOP 01-03 Administrative Review of Applications, a new application must be submitted if the study is ongoing after 5 years from the date of approval. Additionally, any modification to the project must be reviewed and approved by the HRPP prior to implementation. Any failure to adhere to the approved protocol could result in suspension or termination of your project. The HRPP reserves the right, at anytime during the project period, to observe you and the additional researchers ! on this project.

Please note that the MSU HRPP is in the process of seeking accreditation for our human subjects protection program. One of these changes is the implementation of an approval stamp for consent forms. The approval stamp will assist in ensuring the HRPP approved version of the consent form is used in the actual conduct of research. Your stamped consent form will be attached in a separate email. **You must use copies of the stamped consent form for obtaining consent from participants.**

Please refer to your HRPP number (#13-206) when contacting our office regarding this application.

Thank you for your cooperation and good luck to you in conducting this research project. If you have questions or concerns, please contact me at jroberts@research.msstate.edu or call 662-325-2238.

Finally, we would greatly appreciate your feedback on the HRPP approval process. Please take a few minutes to complete our survey at http://www.su! rveymonkey.com/s/YZC7QQD.

Sincerely,

Jodi Roberts, Ph.D.
IRB Officer

cc: Advisor: Diane Tidwell

Informed Consent Form (You must be at least 18 years old to participate)

Title of Research Study: Consumer Acceptance of Sweet Potato Cookies

Study Site: Garrison Sensory Evaluation Laboratory, Mississippi State University

Researchers: Ms. Aja Stokes and Dr. Diane Tidwell. Both are affiliated with Mississippi State University.

Purpose: To determine the acceptability and sensory properties of sweet potato cookies.

Procedures: If you participate in this research, you will be provided with food samples. Please taste them and record your responses on the provided score sheet. This should take about 5-10 minutes. All ingredients of all food items are listed on the sheet posted on the sensory room door as well as on the front desk. All ingredients were purchased locally.

Risks or discomforts: There are no anticipated risks or discomforts. A list of all ingredients is posted on the sensory room door, and the front desk, to prevent a possible food allergy. You may discontinue your participation at any time.

Benefits: Information will be obtained that will help determine the acceptability of gluten-free cookies, which is important for people who are intolerant to gluten or have a wheat allergy.

Incentive to participate: There are no incentives to participate.

Confidentiality: Only the researchers who designed this study will have access to this information. Please note that these records will be held by a state entity and therefore are subject to disclosure if required by law. Research information may be shared with the MSU Institutional Review Board (IRB) and the Office for Human Research Protections (OHRP).

Questions: If you should have any questions about this research project, please feel free to contact Dr. Diane Tidwell at 662-325-0239, dtidwell@fsnhp.msstate.edu. For questions regarding your rights as a research participant, or to express concerns or complaints, please feel free to contact the MSU Regulatory Compliance Office by phone at 662-325-3994, by e-mail at irb@research.msstate.edu or on the web at http://orc.msstate.edu/humansubjects/participant/

Research-related injuries: MSU has not provided for any payment to you or for your treatment if you are harmed as a result of taking part in this study. In addition to reporting an injury to Dr. Diane Tidwell at 662-325-0239 and to the Regulatory Compliance & Safety Office at 662-325-3994, you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the University Police Department at *MSU UNIVERSITY POLICE DEPARTMENT, Williams Building, Mississippi State, MS 39762, (662) 325-2121.*

Voluntary participation: Please understand that your **participation is voluntary.** Your refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue your participation at any time without penalty or loss of benefits. Please take all the time you need to read through this document and decide whether you would like to participate in this research study. If you decide to participate, your completion of the research procedures indicates your consent. Please keep this form for your records.

All ingredients involved in making these food products are approved by the U.S. Food & Drug Administration for human consumption according to their regulations.

_____

Participant Signature Date

_____

Investigator Signature Date

MSU IRB # 13-206
Approved: 07/19/13
Expires: 07/19/18

Diane Tidwell - Study 13-218: Consumer acceptance of sweet potato cookies in pre-school aged children

From:
To:
Date: 7/21/2013 6:45 PM
Subject: Study 13-218: Consumer acceptance of sweet potato cookies in pre-school aged children
CC: , ,

July 21, 2013

Aja Stokes
Dept. of Food Science, Nutrition and Health Promotion
Mississippi State University

RE: HRPP Study #13-218: Consumer acceptance of sweet potato cookies in pre-school aged children

Dear Ms. Stokes:

The initial review of your IRB application referenced above has been completed with the following requested items which must be addressed before the study is approved.

1. Student Committee form
2. Letter from Dr. Tidwell stating that Dr. Briley willl not have access to the data since she is no longer employed by MSU.
3. On the Parental or Legally Authorized Representative Permission the following changes must be made: (1) remove the word template, (2) remove the bolded sentence at the bottom of the page which begins with [** and ends with included:], (3) add the researcher contact information, (4) remove the additional elements section along with #1-7. Just as a general note, I would not have so much! of this bolded.
4. Provide a child assent form for approval.

Please respond to each item by number explaining how it was addressed. You may send the requested items via reply e-mail.

Please ensure that your advisor is on board with your revisions prior to submitting them to me via email.

Please note your study has not yet been approved by the IRB and you may not proceed with the project until it has received approval.

If you have questions or concerns, please contact me at jroberts@research.msstate.edu or call 662-325-2238.

Sincerely,

Jodi Roberts, Ph.D.
IRB Officer

cc: Advisor: Diane Tidwell

You are being asked to allow your child to participate in a research project. This form provides you with information about the project. Please read the information below and ask any questions you might have before deciding whether or not to allow your child to participate.

Title of research project: Consumer Acceptance of Sweet Potato Cookies in Pre-School Aged Children

Site of research project: Food Science, Nutrition and Health Promotion (Herzer)

Name of researcher(s) & University affiliation: Aja M. Stokes (Master of Science Student), Diane K. Tidwell, PhD

The purpose of this research project:

- The purpose of this project is to conduct a sensory analysis on the like or dislike of sweet potato cookies among pre-school aged children.

If you agree to allow your child to participate in this research project, we will ask your child to do the following things:

- The child will be asked to taste two samples; an original recipe of sweet potato cookies containing wheat and a modified recipe of sweet potato cookies containing gluten-free flour. A cup of water will be offered in-between tastings. They will be asked to judge the samples based on a 3-point hedonic scale ranging from like, dislike and neither like or dislike being represented as "smiley faces."

The total estimated time to participate in this research project: 5 minutes

The risks of participation:

- There may be food allergy concerns related to the ingredients that will be used in preparing the samples. See attached form for a complete list of the ingredients that will be used in preparing both samples.
- In addition to reporting an injury to (name of researcher and contact number) and to the MSU Regulatory Compliance Office (662-325-3994), you may be able to obtain limited compensation from the State of Mississippi if the injury was caused by the negligent act of a state employee where the damage is a result of an act for which payment may be made under §11-46-1, et seq. Mississippi Code Annotated 1972. To obtain a claim form, contact the MSU Police Department at *(662) 325-2121.*

The benefits of participation:

- There are no individual benefits for participating in this study.

Compensation:

- There is no compensation for participating in this study.

Confidentiality and privacy protections:

- No personal information regarding the children will be collected at any time during this study (i.e.: names, race etc.). Only ages and gender of

the children will be recorded solely for the purpose of statistical analysis in differentiating between responses based on age and gender.

- Responses to the survey will be locked in a filing cabinet in Room 158 in Herzer building.
- It is important to understand that these records will be held by a state entity and therefore are subject to disclosure if required by law.

Contacts and questions:

- If you have any questions, please ask now. If you should have any questions later or want additional information, please contact Aja Stokes at 662-549-3466 or Diane K. Tidwell at 662-325-0239. For information regarding your rights as a research subject, please contact the MSU Regulatory Compliance Office at 662-325-3994.

If you do not want your child to participate:
Please understand that your child's participation is **voluntary.** Your refusal to allow your child to participate will involve **no penalty** or loss of benefits to which you or your child is otherwise entitled. You may discontinue your child's participation **at any time** without penalty or loss of benefits. Your child may skip any items that he or she chooses not to answer. Your refusal will not impact current or future relationships with Mississippi State University. To do so, simply tell the researcher that you wish to stop.

If after reading the information above, you agree to allow your child to participate, please sign below. If you decide later that you wish to withdraw your permission, simply tell the researcher. You may discontinue your child's participation at any time. You will be given a copy of this form for your records.

Child's name (please print)

_____

_____ _____
Parent or *Legally Authorized Representative's Signature Date

_____ _____
Investigator's Signature Date

If a Legally Authorized Representative (rather than a parent), must have documentation to show LAR status.

MSU IRB# 13-218
Approved: 07/24/13
Expires: 07/24/18