Beta-carotene Retention and Changes in Nutrient Composition and Antinutrient Level of Traditional Foods Prepared from Yellow Maize Variety from Ethiopia

By

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ABSTRACT

*Vitamin A deficiency (VAD) is a public health problem in Ethiopia. It affects vision, growth, tissue differentiation, reproduction and immune system. Yellow maize varieties are known to contain high amount of β-carotene and other carotenoid. This study was designed to determine β-carotene retention and changes in nutrient composition and antinutrient level during traditional foods prepared from yellow maize. In addition, β-carotene retention in and acceptability of traditionally prepared foods from a yellow maize variety of high β-carotene content were investigated. Maize varieties were collected from Ethiopian agricultural research institute (Melkasa and Bako center) and Ethiopian seed enterprise (Bahir Dar and Gibie Awash branch). Total carotenoid and β-carotene level were investigated by UV Spectrophotometer (*Agilent 8653*) and high performance liquid chromatography, respectively. The nutrient (protein, fat, fiber, ash, carbohydrate, Zn, Fe, Ca) and antinutrient content in raw maize varieties and corresponding traditional foods were determined. In addition, β-carotene level was determined in these foods and their degree of retention was calculated. Sensory quality of the two traditional foods (stiff porridge and unleavened flat bread) was evaluated using seven hendonic scale. The total carotinoid content in the yellow maize varieties ranged from 11.4 (Melkassa 7) to 28.9 µg/g (Melkassa 1).The β-carotene ranged from 1.2 (Gibie Awash) to 3.1 µg/g (Melkasa 1). No carotenoid was detected in the white maize variety (BH 660). There was significant difference ($P<0.05$) among maize varieties in total caroteniod and β-carotene level. Yellow maize variety contains high amount of β-carotene and total carotenoids than white variety. In addition, its other nutrients and bioavailability of Zn and Fe were comparable to white maize variety. β- carotene level retention in traditional foods was ranged 55.9% for besso to 88.9% for stiff porridge. The level of retention was significantly different ($P<0.05$) among traditionally prepared foods. Changes in Proximate and mineral composition and antinutrient content of the six type of traditionally processed foods differed significantly ($P<0.05$) from raw maize (melkasa 1). The selected foods for sensory acceptance analysis stiff porridge (88.9%) and unleavened flat bread (88.3) had highest β-carotene retention. Foods prepared from yellow maize variety were acceptable sensory wise. Promotion of yellow maize varieties is vital to enhance Vitamin A intake and reduce risk of diseases such as cardiovascular and cancer without altering intake of other nutrients.*

Key words:- VAD, β-carotene, Maize, Retention

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ABRIVATIONS

ANOVA:	Analysis of Variance
AAS:	Atomic Absorption Spectrophotometer
BHT:	Butylated Hydroxytoluene
CIMMYT:	International Maize and Wheat Improvement Center
DMSO:	Dimithyl Sulfoxide
DW:	Dry weight
ES:	Ethiopian Standard
FAO:	Food and Agricultural Organization
FMOH:	Federal Ministry of Health
HPLC:	High Performance Liquid Chromatography
ISO:	International Organization for Standardization
MI:	Micronutrient Intuitive
ODS:	Octadecy-Silca
PTFE:	Polyterrafluoroethylene
RDA:	Recommended Daily Allowance
RBP:	Plasma Retinol Binding Protein
RE:	Retinol Equivalents
SCN:	United Nations System Standing Committee on Nutrition
SNNP:	Southern Nation and Nationalities and People
SPSS:	Statistical Package for the Social Sciences
UNICEF:	United Nations Children's Fund
USDA:	United State Agency for International Development
VAD:	Vitamin A Deficiency
WFP:	World Food Program
WHO:	World Health Organization

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1. INTRODUCTION

Micronutrient deficiency is a global problem even much bigger than protein energy malnutrition. More than 2 billion people are affected globally by 'hidden hunger' particularly due to the deficiency of vitamin A, iodine, iron and zinc. Most of these people live in low income countries and are typically deficient in more than one micronutrient (WHO/WFP/UNICEF, 2006; Thompson and Amorson, 2014). Micronutrient deficiency imposes enormous costs on societies in terms of ill health, lives lost, reduced economic productivity and poor quality of life (Ruel, 2001).

Inadequate dietary intake is primary causes of Vitamin A deficiency (VAD) in developing countries (Adem et al., 2012). Besides the increased risk of mortality, VAD limits growth, weakens immunity, causes blindness and impairs the normal development of healthy skin and tissues (WHO/FAO, 2006).

Vitamin A deficiency (VAD) predisposes an estimated 100 million Africans to a higher risk of visual impairment and blindness (African Union, 2005). Vitamin A deficiency is a serious public health problem in Ethiopia. National prevalence rates of 1.7% for Bitot's Spots and 0.8% of night-blindness among children and 1.8% for night-blindness among mothers. Nationally, 37.7% of children had deficient serum retinol levels (Demissie et al., 2010).

Addressing the global challenge of micronutrient deficiency requires the need for many strategies both short and long-term sustainable approaches. In addition to micronutrient supplementation and fortification, promoting food based approaches is important to enable adequate intakes of micronutrients by much of the population. Agricultural biotechnology offers the opportunity of increasing crop yields and improvement the micronutrient content of staple foods that poor people already eat, and provide a comparatively inexpensive, cost effective, sustainable and long term means of delivering micronutrients to the poor (Thompson and Amoroso, 2011, 2014). However, currently implementing strategy in Ethiopia is supplementation of vitamin A capsule for children 6

to 59 month of age which is expensive and not sustainable when compared to food base approach (Birara from FMOH, Personal communication).

In Ethiopia, maize is a staple food in major maize producing areas. The per capita consumption of maize in Ethiopia is about 60 kg per annum (Mosisa et al., 2011). Foods of plant origin are an important source of pro-vitamin A in developing countries (Tumuhimbise et al., 2013). Yellow maize, in addition to being dietary source of energy, lipids, protein, minerals and vitamins, it is a source of carotenoids (Menkir et al., 2008). Carotenoids are a diverse family of yellow-orange pigments generally categorized into two groups; carotenes (eg. β-carotene, α-carotene) and xanthophylls (eg. β-cryptoxanthin, lutein, zeaxanthin). β-carotene, α-carotene and β-cryptoxanthin are important precursors of vitamin A in humans (Tanumihardjo, 2002). Carotenoids are also potent antioxidants and important physiological modulators (Yeung and Laquatra, 2003).

Yellow maize varieties contain between 0.25 and 2.5 μg/g dry weight (DW) of pro-vitamin A, while deep yellow or orange varieties may contain up to 15 μg/g (DW) of pro-vitamin A carotenoid (Nuss & Tanumihardjo, 2010). However, white maize varieties are dominantly consumed in Africa (M'mboyi et al., 2010) which are devoid of pro-vitamin A carotenoids. This may partly explain why VAD is a major public health problem in sub-Saharan Africa (Nuss and Tanumihardjo, 2010). Yellow maize consumption decreases vitamin A deficiency among vulnerable groups (Muzhingi et al., 2008). Thus consumption of such varieties is important for prevention and control of VAD particularly in countries such as Ethiopia where the problem is public health significance. However, attributed to their color, acceptability of yellow maize varieties in the market is low.

Consumption of maize requires pre-treatments such as heat processing, which could confer some nutritional benefits, as well as alter the physicochemical contents and properties of its components (Siljestrom et al.,1986 as cited in Oladeji et al., 2015). In the whole crop, the carotenoid molecules are less susceptible to degradation because they are protected within tissues. Carotenoids can loss due to processing that dislocates the

plant matrix including the cellular compartments and binding proteins that serve to protect and stabilize the carotenoid pigment (Aman et al., 2005). It is important to quantify the losses of provitamin A carotenoids during processing of maize. Thus, the present study designed to determine β-carotene retention and change and antinutrient level of traditional foods prepared from yellow maize variety. In addition sensory evaluation was conducted for selected foods with better β-carotene retention as well.

2. OBJECTIVES

2.1 General Objective

To determine β-carotene retention and changes in nutrient composition and antinutrient level of traditional foods prepared from yellow maize variety from Ethiopia

2.2 Specific Objective

- To determine level of total carotenoids and β-carotene in some common maize varieties in Ethiopia
- To investigate β-carotene retention in traditionally prepared foods from the selected yellow maize variety
- To determine nutrient composition and changes during traditional food preparation
- To evaluate the sensory properties of foods prepared from the selected yellow maize variety

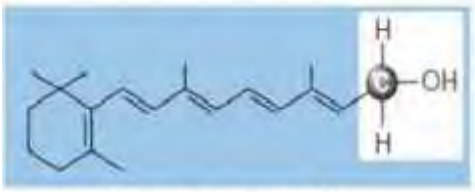
3. LITERATURE REVIEW

3.1 Deficiency of Micronutrients

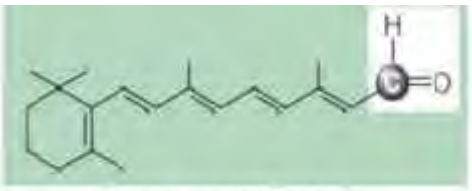
Micronutrients are an essential nutrient, as a trace mineral or vitamin that is required by an organism in minute amounts that enable the body to produce enzymes, hormones and other substances essential for proper growth and development. Even though they are needed in tiny amounts, consequences of their absence are severe (Berdanier, 1998). More than 2 billion people in the world suffer from micronutrient deficiencies caused largely by a dietary deficiency of vitamins and minerals especially iodine, iron, vitamin A and zinc (Thompson and Amorson, 2014). Micronutrient deficiencies account for about 7.3% of the global burden of disease, with iron and vitamin A deficiency ranking among the 15 leading causes of the global disease burden. Although people in all population groups in all regions of the world may be affected, the most widespread and severe problems are usually found amongst resource poor, food insecure and vulnerable households in developing country. The public health importance of these deficiencies lies upon their magnitude and their health consequences as they affect fetal and child growth, cognitive development and resistance to infection. In addition to the obvious and direct health effects, the existence of micronutrient deficiency has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation (Allen et al., 2006).

3.2 Vitamin A

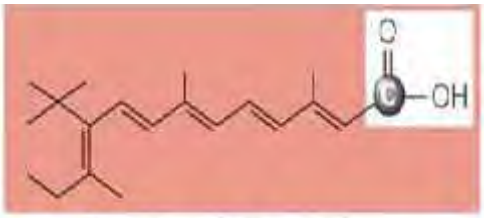
Vitamin A is a fat soluble vitamin which can be found in body in three main active forms that are retinol, retinal and retinoic acid, collectively, these compounds are known as retinoids. The cells in the body can convert retinol and retinal to the other active forms of vitamin A as needed. The conversion of retinol to retinal is reversible; whereas the further conversion of retinal to retinoic acid is irreversible. Foods derived from animals provide compounds retinyl esters that are readily digested and absorbed as retinol in the intestine. Foods derived from plants provide carotenoids, some of which have vitamin A



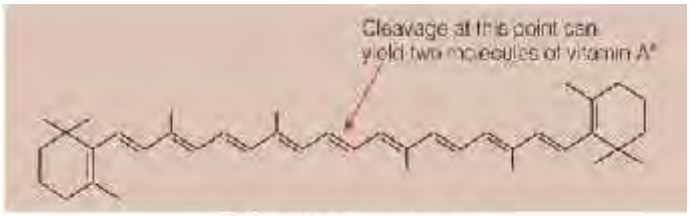
Retinol, the alcohol form



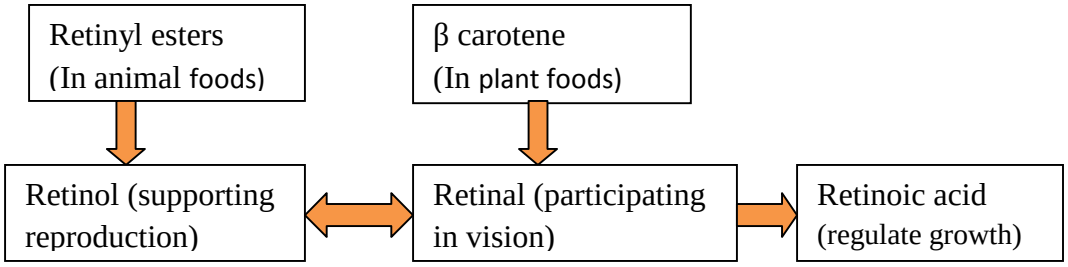
Retinal, the aldehyde form



Retinoic acid, the acid form



Beta-carotene, a precursor



vitamin A. However, other carotenoids, such as lycopenene and lutein, are devoid of provitamin A activity (Yeung and Laquatra, 2003).

The β-carotene content of food varies with the growing conditions and the post-harvest storage of the food. The bioavailability and bioconversion of provitamin A carotenoids can be influenced by various factors such as the digestibility of the food, molecular linkage, amount of carotenoids consumed in a meal, matrix in which the carotenoid is incorporated, intake of dietary fat, type and amount of fiber, alcohol, nutritional status of the individual as well as genetic and host-related factors (Berdanier, 1998; Jim and Truswell, 2002).

Table 1: Carotenoids with vitamin A activity (Berdanier 1998)

Compound	**Relative Potency**
β-Carotene	100
α-Carotene	53
γ-Carotene	43
Cryptoxanthin	57
Lycopene	0
Zeaxanthin	0
Xanthophyll	0

3.2.1 Metabolism

Dietary sources of vitamin A that are retinyl esters and retinol from certain animal tissue and β-carotene from certain plants are hydrolyzed in the intestinal mucosa, releasing retinol and free fatty acids. Retinol derived from esters and from the cleavage and reduction of carotenes is re-esterified to long chain fatty acids in the intestinal mucosa and secreted as a component of chylomicrons into the lymphatic system. Retinyl esters contained in chylomcron remnants are taken up by and stored in the liver. Retinol is released from the liver when it is needed and transported to extrahepatic tissues by the

plasma retinol binding protein (RBP). The RBP complex attaches to specific receptors on the surface of the cells of peripheral tissues (Sommer, 1995; Harvey and Ferrier, 2011).

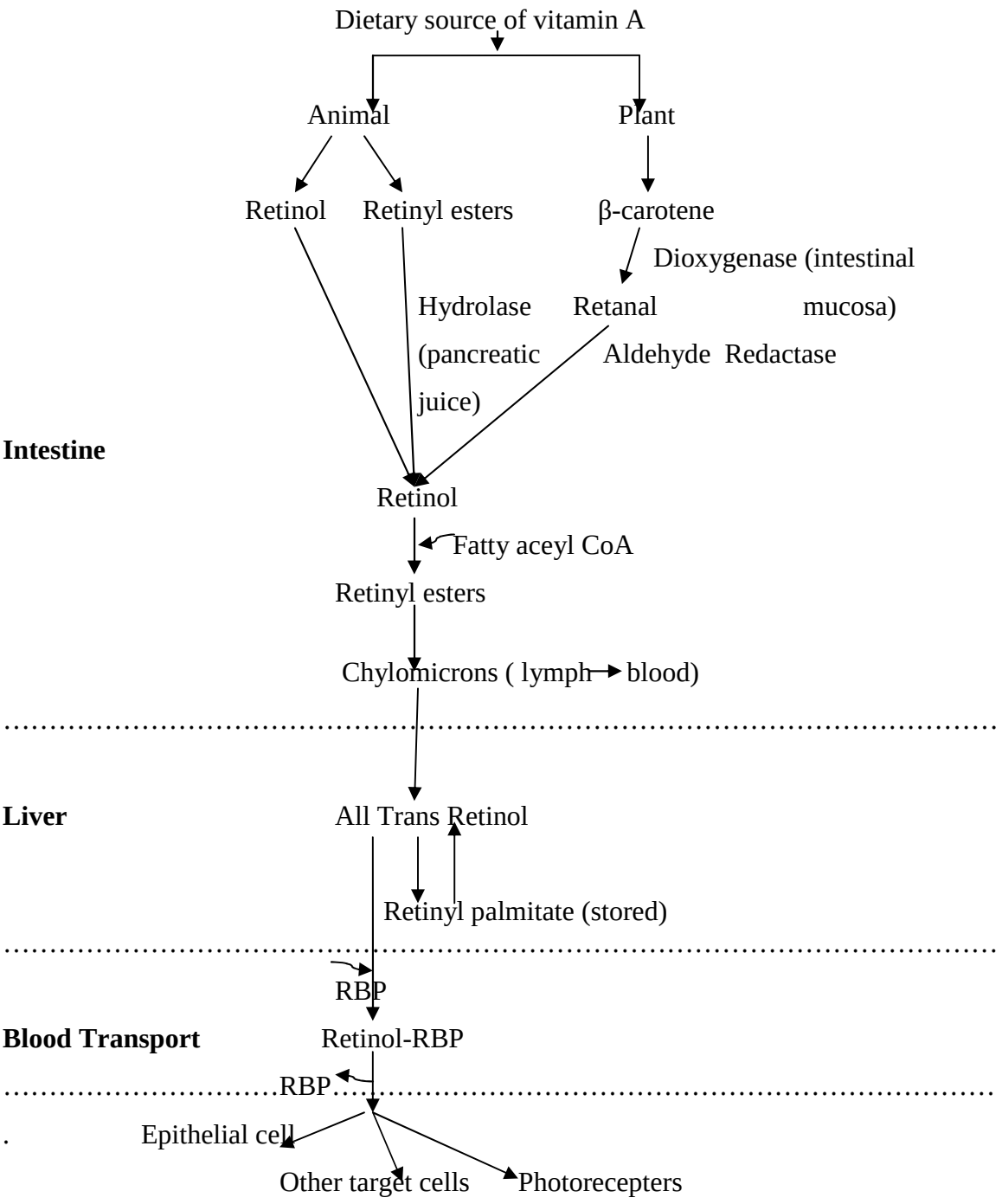


Figure 3: Scheme of Vitamin A Metabolism (Sommer, 1995; Harvey and Ferrier, 2011)

3.2.2 Biological Significant of Vitamin A

Vitamin A and its precursor, pro-vitamin A such as β-carotene, have diverse role and profound effect on heath. Its major roles are promoting vision, participating in protein synthesis and cell differentiation, marinate the health of epithelial tissues and it is known as the anti-infective vitamin, because it is required for normal functioning of the immune system (Yeung and Laquatra, 2003; WHO/FAO 2004; Whitney and Rady, 2008). According to Whitney and Rady (2008) the three forms of vitamin A carry out specific functions. Retinal is active in vision and it is also an intermediate in the conversion of retinol to retinoic acid. Retinoic acid acts like a hormone, regulating cell differentiation, growth and embryonic development. Retinol supports reproduction and it is a major transport and storage form of vitamin A.

β-carotene and other carotenoids are also potent antioxidants and important physiological modulators (Yeung and Laquatra, 2003). Antioxidants have proven effective in fighting free radicals, highly unstable compounds that are formed when oxygen combines with certain substances. Free radicals can damage the basic structure of cells and thus lead to chronic diseases such as cardiovascular disorder and cancer and accelerate the aging process. Thus, β-carotene and other carotenoids protect oxidation and free radical damage by quenching singlet oxygen (Mozaffarieh et al., 2003; Sesso et al., 2004).

3.2.3 Recommended Intake

Daily nutritional needs in vitamin A for different class-ages were evaluated by FAO/WHO to tackle vitamin A deficiency. The mean requirement intake is the minimum intake to prevent xerophthalmia in the absence of clinical or sub-clinical infection that expressed as µg retinol equivalents (µg RE). This intake should account for the proportionate bioavailability of performed vitamin A (about 90%) and provitamin A carotenoids from a diet that contains sufficient fat (at least 10g daily) whereas the recommended safe level intake is the average continuing intake of vitamin A to permit adequate growth and other vitamin A dependent functions and to maintain an acceptable total body reserve of the vitamin (WHO/FAO, 2004). As the body can derive vitamin A

from various retinoids and carotenoids, its contents in foods and its recommendations are expressed as retinol activity equivalents (RAE) (Whitney and Rady, 2008). On a study (Ejigui et al., 2005) conversion factor for beta carotene in maize expressed 1RE=3.33 International Unit (IU); 1IU= 0.60 µg β carotene; 1RE= 3.33x0.6µg β-carotene approximately 2 µg β-carotene.

Table 2: Estimated mean requirement and safe level of intake for vitamin A, by group (WHO/FAO 2004)

Group	**Age**	**Mean requirement (µg RE/day)**	**Recommended safe intake (µg RE/day)**
Infants and children	0-6 months	180	375
	7-12 months	190	400
	1-3 years	200	400
	4-6 years	200	450
	7-6 years	250	500
Male	11-12	500	1000
	13-15	600	1000
	Adult (15)	600	1000
Adolescents	10-18 years	330-400	600
Adults: Female	19-65 years	270	500
	65+years	300	600
Male	19-65 years	300	600
	65+years	300	600
Pregnant women	-	370	800
Lactating women	-	450	850

3.2.4 Vitamin A Deficiency and Its Prevalence

VAD is defined as liver stores below of retinol 20 µg/g (0.7µmol/g). Serum retinal levels may still be within the homostatically regulated normal range. By convention, serum retinol levels <20 µg/dL(0.70 µmol/L) are considered deficient (SCN 2004). The main

cause of VAD in developing countries is inadequate dietary intake of vitamin A and its precursor carotenoids. The consumption of vitamin A rich foods is affected by many factors like inadequate production of vitamin A rich foods, unavailability of vitamin A rich foods in the markets, large family size, high maternal parity levels, land size, that are presumed to contribute to inadequate consumption of vitamin A rich foods in developing countries (Demissie et al., 2009). The secondary cause of vitamin A deficiency include retinol is absorbed from the small intestine dissolved in lipid. In people with a very low fat intake (less than about 10% of energy from fat), absorption of both retionol and carotene is impaired, and low fat diets are associated with vitamin A deficiency (Yeung and Laquatra, 2003; Gibney et al., 2009).

VAD is one of the most prevalent forms of micronutrient deficiency in the world. The first symptoms of vitamin A deficiency are night blindness and drying of the conjunctiva of the eye. Bitot's spots may be presented in the cornea. With continued vitamin A deficiency, progressive damage to the eye results from drying of the cornea and irreversible corneal damage resulting in xerophthalmia, keratomalacia, and blindness (McGuire and Beerman, 2011).

Globally 190 million (33.3%) children under the age of 5 years old are vitamin A deficient, with Africa having one of highest prevalence, at 44.4 % (WHO, 2009). VAD is the cause for 1.2 to 3 million children and significant numbers of women to die, and 4.4 million children and 6.2 million women suffer from xerophtalmia (SCN, 2004).

Deficiency of vitamin A is a major cause of blindness in developing countries and also contributes to impaired immune function resulting in increased mortality (Sanders and Emery, 2003). Africa and Southeast Asia have the highest burden of vitamin A deficiency (WHO, 2009). Estimated 250,000–500,000 vitamin A-deficient children become blind every year, approximately half of which die within a year of becoming blind. (WHO/FAO, 2006).

VAD is public health problem in Ethiopia. In Ethiopia pregnant women, infants and young children are most susceptible for VAD and sadly, its deficiency affects about 7.7

million children and results in an estimated 50,000 deaths each year (FMOH 2011 as cited in Owen Fofanh et al., 2011).

3.2.5 Strategies to Combat Vitamin A Deficiency

Globally there are short and long term strategies to combat micronutrient deficiency that are supplementation, and food-base approaches such as food diversification, fortification (exogenous fortification and biofortification). Food-base approaches promote the consumption of foods that are naturally rich in micronutrient or are enriched foods through fortification (Thompson and Amoroso, 2011).

Supplementation is the term used to describe the provision of relatively micronutrients, usually in the form of pills, capsules or syrups. It has the advantage of being capable of supplying an optimally amount of a specific nutrient or nutrients in a highly absorbable form to control in individuals or population groups that have been identified as being deficient (Allen, 2006). Although supplementation has saved many lives and much suffering has been avoided as a result of these efforts, it is a short term emergency measure. It fails to recognize the root cause of micronutrient deficiency and to assist communities and households to feed and nourish themselves adequately (Ruel, 2011). Supplementation usually requires the procurement and purchase of micronutrients in a relatively expensive pre-packaged form and effective distribution system. It cannot provide the overall long term economic benefits and sustainability that food base approaches can deliver (Allen, 2006).

Dietary diversification is a long term strategy that complements supplementation and fortification programs. It refers to a variety of strategy that aim to increase the production, availability and access to foods rich in micronutrients and the bioavailability of micronutrients from the diet. Dietary diversification can be achieved through horticultural approaches such as home gardens, behavioral change and improved methods of food preparation and preservation that minimize the loss of micronutrients (Ruel, 2001).

Exogenous fortification is adding of essential vitamin and minerals to foods which are regularly consumed such as flour, salt, sugar and cooking oil (Nuss and Tanumihardjo 2010).In many situations, this strategy can lead to relatively rapid improvements in the micronutrients status of a population, and at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks (Allen, 2006).

Biofortification is the process of breeding nutrients into crops through conventional and transgenic methods (Saltzman et al., 2014). It is a promising strategy for combating hidden hunger that provides a sustainable, long term strategy for delivering micronutrient comparatively inexpensive and cost effective way to rural populations in developing countries who may have limited access to diverse diets, supplements and commercially fortified foods. Unlike the continual financial outlays required for supplementation and commercial fortification programs, a onetime investment in plant breeding can yield micronutrient rich planting materials for farmers to grow for years to come (Bious and Welch, 2010; Thompson and Amoroso, 2014).

Ethiopia currently implemented short term strategy which is the primary prevention strategy which forms part of the routine immunization program, the integrated management of childhood illnesses that is providing vitamin A supplementation to children aged 6-59 months. It is expensive and not sustainable. This program does not address women in the post partum period. Implementation of other strategies is not still started but preparation of implementation guidelines is on process (Birara from FMOH, Personal communication). A study is going on at Mekelle University on orange flesh potato to use it as one of the food base approach to control vitamin A deficiency (Abenet from MNI, Personal communication).

3.2.6 Stability of provitamin A carotenoids

Provitamin A carotenoids are easily destroyed by exposure to light, oxygen and during processing, heating and storage (Rodriguez-Amaya 1997). In home preparation, losses of carotenoids generally increase in the following order: microwaving< steaming < boiling

< sautéing. Deep-frying, prolonged cooking, combination of several preparation and cooking methods, and pickling all result in substantial losses of carotenoids. Whatever the processing method, carotenoid retention decreases with longer processing time, higher processing temperature, and cutting or puréeing of the food. Retention is significantly improved by cooking with the lid on, reducing the processing time, lowering the temperature, and shortening the time lag between peeling, cutting, or puréeing and processing (Rodriguez-Amaya and Kimura, 2004).

3.3 Maize

3.3.1 Production and Productivity

The way maize has spread globally from its origin in America indicates its remarkable adaptability. Unlike wheat and rice, which are limited by climatic conditions, maize flourishes in a variety of different soils, latitudes, altitudes and weather conditions (Bhupender et al., 2012). Although maize can be cultivated in many different regions (eg. equatorial forest or savannah), the savannah is the most favorable climatic zone, with an average annual rainfall of between 800 and 1200mm and strong sunlight which will help to reduce parasitism (Hoopen and Maiga, 2012).

Maize is cultivated globally on more than 160 million hectare area across 166 countries (Bhupender et al., 2012). Developing countries plant two thirds of the global maize production while industrialized countries plant one third (M'mboyi et al., 2010).Maize is the most heavily cultivated cereal crop globally, with an average annual production of around 817 million tons in 2009, and followed by wheat (681 million tonnes) and rice (678 million tonnes) (Hoopen and Maiga, 2012). Maize provides a high yield of food energy at a relatively lower expense of seed and labor (Bhupender et al., 2012).

Nine of the top 25 maize-producing countries are from Africa, producing 17.4 Million hectare which is 12.5% of the maize global area (James, 2003). It is planted annually on over 15.5 million hectares of land in East and Southern Africa (ESA) (M'mboyi et al., 2010).

Ethiopia is among the major maize producers in the world and ranked fourteenth and third in Africa next to South Africa and Nigeria (FAO, 2012).In Ethiopia the crop is widely cultivated at altitudes ranging from 1500–2200 meters above sea level of Western, Southwestern, and Southern parts of the country. Maize production takes significant share of cereals and grain in any production year. Among cereals, maize ranked second to tef in area coverage (21.7% for maize and 27.4% for tef), and first in total production (28.5% for maize and 19.9% for tef) and productivity (Mosisa et al., 2011). Three regional states including Oromia, Amhara and SNNP contribute to 94% of the total annual production (Ethiopia Commodity Exchange Authority, 2009).

3.3.2 Consumption

Cereals have been part of the human diet since prehistoric times. Maize is the most widely grown and consumed staple crop in Africa with more than 300 million Africans depending on it as their main food source. Maize is the staple food crop for over 24 million households in East and Southern Africa (ESA). It is the most important crop for households both consumption, (accounting for 62% of all household cereal consumption), and as source of cash income (accounting for about 54% of cash income) (Berhanu, 2011). The average maize consumption in Africa is 106.2g/person/day which is far greater than other maize-consuming regions such as Europe, the Far East, Latin America or the Middle East (WHO, 2003). In Ethiopia three quarters of maize produced is used for household consumption, only about ten percent is marketed and the remainder is used for seed, in kind payments for labor, and animal feed (Schneider and Anderson, 2010).

Maize utilization is to some extent related to the grain types. 85% of global maize consists of yellow endosperm, 10-12% is of white endosperm, and 5-8% is of red, blue, purple, or black kernels. Maize of all grain types and colors are found in landraces in Sub-Saharan Africa, but the predominant ones are white dents (M'mboyi et al., 2010).

Yellow maize is basically similar to white maize except for its grain color which has yellow to orange shades of color due to the presence of chemical compounds known as carotenoids, mainly in the endosperm. Yellow maize is usually preferred to white maize

all trans-β-Carotene

all trans-α-Carotene

all trans-β-Cryptoxanthin

carotinoid and β-carotene, β-cryptoxanthin and α-carotene, which are the three fractions of carotenoids showing pro-vitamin A activity (Rios et al., 2014).

Safawo et al (2010) evaluated the total carotenoid and β-carotene content of sixty four maize varieties that grown in India by using UV/Vis spectrophotometer and high performance liquid chromatography (HPLC) respectively. The finding indicated that averaged total carotenoids were 14 µg/g (5.58 to 63.9 µg/g) and β-carotene 1.69 µg/g (0.122 to 4.74 µg/g). Dixon et al (2000) determined total carotenoid content of 16 improved yellow maize varieties and reported the result ranged 143 to 278 µg/g. Drinic et al (2014) reported the β-carotene content of 17 maize genotypes ranged from 8.69 to 21.90 mg/kg.

Studies have also demonstrated the promising bioavailability of provitamin A in maize (Howe and Tanumihrdjo, 2006; Kean et al., 2008; Li et al., 2010). Muzhingi et al (2011) study found that in Zimbabwean men, 300 g cooked yellow maize containing 1.2 mg β-carotene that was consumed with 20.5 g fat showed the same vitamin A activity as 0.38 mg retinol and provided 40–50% of the adult vitamin A RDA. However, in Africa white maize which lack provitamin A carotinoid is preferred for human consumption and it is often the first solid food given to African infant (Faber et al., 2005 as cited in Li et al., 2007).

De Groote and Kimenju (2008) showed consumer preference for yellow maize in Kenya that result indicated strong preference was showed for white maize by consumers. Only a minority would buy yellow maize at the same price as white maize, on average, consumers need a price discount of 37% to accept yellow maize. In addition the report showed that in Kenya poor acceptance of yellow maize comes from prejudice and negative associations, such as food aid and animal feed, rather than from sensory characteristics such as taste.

3.3.4 Nutrients other than Carotinoid in Maize

Cereals and cereal products are an important source of energy, carbohydrate, protein and fiber. They also contain a range of micronutrients such as vitamin, sodium, magnesium and zinc (McKevith, 2004; Belfield and Bronwn, 2008). Maize is a vehicle for vitamin and mineral deficiency intervention (Gwirtz and Garcia-Casal, 2014). The chemical composition of the maize kernel and its nutritional value give it a good position among the group of cereals in the "agrifood" category (Hoopen and Maiga, 2012).

Table 3: Proximate and mineral composition of common maize per 100g (USDA National Nutrient Database for Standard References)

	Yellow maize	**White maize**
Proximate		
Water (g)	10.37	10.37
Energy (kcal)	365	365
Protein (g)	9.42	9.42
Fat (g)	4.74	4.74
Ash (g)	1.2	1.2
Carbohydrate (g)	74.26	74.26
Fiber (g)	7.3	7.3
Mineral:		
Calcium (mg)	7	7
Iron (mg)	2.71	2.71
Zinc (mg)	2.21	2.21
Potassium (mg)	287	287
Copper (mg)	0.31	0.31
Phosphorus (mg)	210	210
Magnesium (mg)	127	227
Sodium (mg)	35	35
Manganese (mg)	0.48	0.48
Selenium (µg)	15.5	15.5

Table 4: Proximate and mineral composition of maize varieties found from different research report

	Range Reported by					
	Ndukwe et al (2015)	Kumar and Kweera (2013)	Abdo et al (2013)	Ullah et al (2010)	Ijabadeniyi And Adebolu (2005)	Dixon et al (2000)
Proximate						
Moisture (%)	9.9-11.4	8.96-12.5	12.6-13.6	9.2-10.9	10.7-11.8	-
Energy (kcal)	-	398.2-429.1	-	307.0-394.1	-	-
Protein (%)	10.72 -12.33	8.7-8.96	7.8-11.6	7.7-14.6	10.7-11.3	-
Fat (%)	3.2-4.1	3.9-4.2	3.0-3.2	3.2-7.7	4.8-5.0	3.1 - 7.1
Ash (%)		1.1-1.3	1.7-3.1	0.7-1.3	1.3-3.7	-
Carbohydrate (%)	68.7-72.2	-	-	69.7- 74.5	65.6- 70.2	-
Fiber (%)	1.84 -2.06	2.1-2.4	3.1-3.9	0.8-2.3	2.1-2.8	-
Mineral:	-	-	-	-	-	-
Calcium (mg)	163.8-180.7	44.0-58.0	183.3-233.3	41.0-59.0	-	-
Iron (mg)	2.8 - 3.5	-	-	3.8-5.6	-	1.4 – 15.9
Zinc (mg)	-	-	-	3.7-5.2	-	1.2 – 9.7
Potassium (mg)	315.7-342.8	-	126.7-376.7	291.5-347.1	-	-
Copper (mg)	-	-	-	1.1-1.4	-	-
Phosphorus (mg)	275.3-285.8	245-280	815.64-2103.3		-	230-400
Magnesium (mg)	-	-	51-103	98.5-112.5	-	-
Sodium (mg)	61.8 -180.9	-	230-383	54.0-62.0	-	-

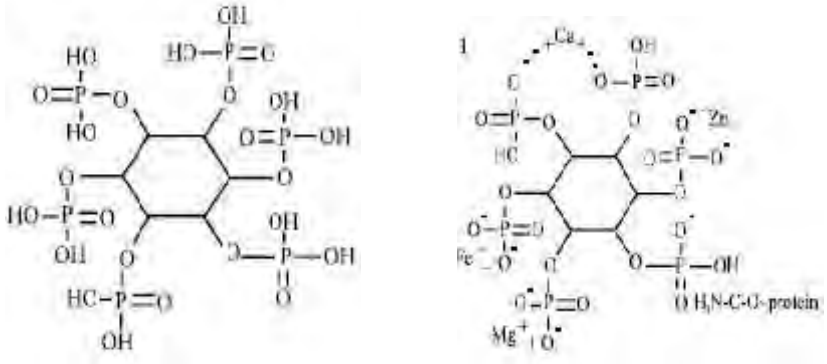


Figure 5: Structure of phytic acid and its possible interaction with both metal cations (minerals) as with protein residues.(Coulibaly et al., 2011)

3.3.6 Traditional Foods from Maize in Ethiopia

Ethiopian consumes maize by preparing different dishes. These include bread, injera, porridge, boiled maize, roasted maize, besso, kitta, kinche, Nefro, Soup, Salad and local beer (Asrat, 2011). About 93 and 92% of the maize growers make bread and injera from maize, respectively. About 83% of the farmers consume boiled maize, 72% make thick porridge and about 64% use maize to make the local beer, tella. Hence, in order of importance, maize is used for making bread, followed by injera, boiled maize, thick porridge and local beer (Berhanu, 2011).

3.3.7 Retention of Beta Carotene in Maize Product Foods

Li et al (2007) evaluated β-carotene losses as percentage of the initial β-carotene content in the raw whole kernels by using maize with high beta carotene content (10.49 $\pm$ 0.2µg/g) to prepare traditional foods (fermented and unfermented porridges). As the finding indicated losses of β-carotene during the preparation of fermented and unfermented porridges were 24.5% and 24.8% respectively and the loss was not significantly different from raw maize. According to Ejigui et al (2005), who studied the benefit change and drawback of traditional fermentation (4 days) on nutrients and anti-nutritional factors of yellow maize, the result exhibited a significant decrease in crude protein (9%), crude fat (11%), total ash (54%), calcium (38%), Retinol Equivalent (RE) (66%) and a significant increase in carbohydrate and fiber (9%), antinutritional factor phytates decreased by 61.5%; Lose of Zinc (7.64%) and Iron (2.79%) was not affected significantly. On the other hand Muzhingi (2008) reported on the effect of cooking yellow maize by boiling at 100^0c for 30 minutes increased carotinoid concentration but baking at 450^0c for 25 minutes decreased carotenoids concentration by almost 70% that compared to raw maize flour.

Kavitha and Parimalavalli (2014) studied the effect of roasting and germination on proximate composition of cereal and legume flours. The result indicated that there was significant difference between raw maize and processed in proximate composition.

Roasting decreased all proximate composition (ash, fat, protein, fiber, carbohydrate and energy) and germination increased moisture and protein content.

4 MATERIALS AND METHODS

4.1 Location of the Study and Source of Raw Materials

The experiment was conducted in Addis Ababa, Ethiopia which lies at an altitude of 2,500 meter and is located at 9.03^0N 38.74^0E. Analysis of total carotenoid and beta carotene were conducted at the chemical testing laboratory of Ethiopian Conformity Assessment Enterprise. Maize flour preparation and sensory property of foods were done at the laboratory of Addis Ababa University, Food Science and Nutrition Center.

Seed of 2 yellow maize varieties (Gibie Awash, CML165) were collected from Bako Agricultural Research center. Bako located at longitude and latitude of $9^0$08N $37^0$03E, at altitude of 1590 meters and its distance from Addis Ababa is 250km. 3 yellow maize varieties (Melkasa -1q, Melkasa-7, Melkasa-1) were obtained from Melkasa Agricultural Research Center. Melkasa located at latitude of 8^0 24N, longitude of $39^0$21, at altitude of 1550 meters and its distance from Addis Ababa is 115km. 1 yellow (CML 161) and 1 white maze variety (BH 660, which is dominantly cultivated in the country) were collected from Ethiopian Seed Enterprise, Bahir Dar branch. Bahir Dare located at latitude of $11^0$36N longitude of 37 37°23E, at altitude of meters 1800 meters and distance from Addis Ababa 578km. BHQPY 545 was obtained from Ethiopian Seed Enterprise, Gibie Awash branch. Other ingredient baking powder and shorting were purchased from local market in Addis Ababa.

Figure 6: Kernel color of eight maize varieties

4.2 Preparation of Stiff porridge, Besso, Anebabero, Unleavened flat bread and Nifro

Stiff porridge: Maize grain was cleaned and milled into fine flour and it was prepared by mixing 200g flour in 350ml boiling water and cooked at 100^0c until it retains the right consistency and flavor that took 20 minute.

Anebabero: maize grain was cleaned manually and milled into flour. 1 kg flour was mixed with 1600ml water and 1 spoon ersho (starter) and kneaded until the dough was formed. Then it was baked into anebabero immediately after fermentation. The time taken for fermentation was 30 minute.

Unleavened flat bread (Kitta): it is an unfermented product. Maize grain was cleaned and milled into fine flour. 125g maize flour was mixed with 200 ml water and baked.

Besso (roasted maize flour): Maize grain was roasted at 150^0c and milled into flour and 125g was mixed thoroughly with water 250ml.

Nifro: Dry 250g of whole maize grain was soaked for 12 hour then boiled at 100^0c in a pot until it was soft and that was taken 2 hour and 15minutes.

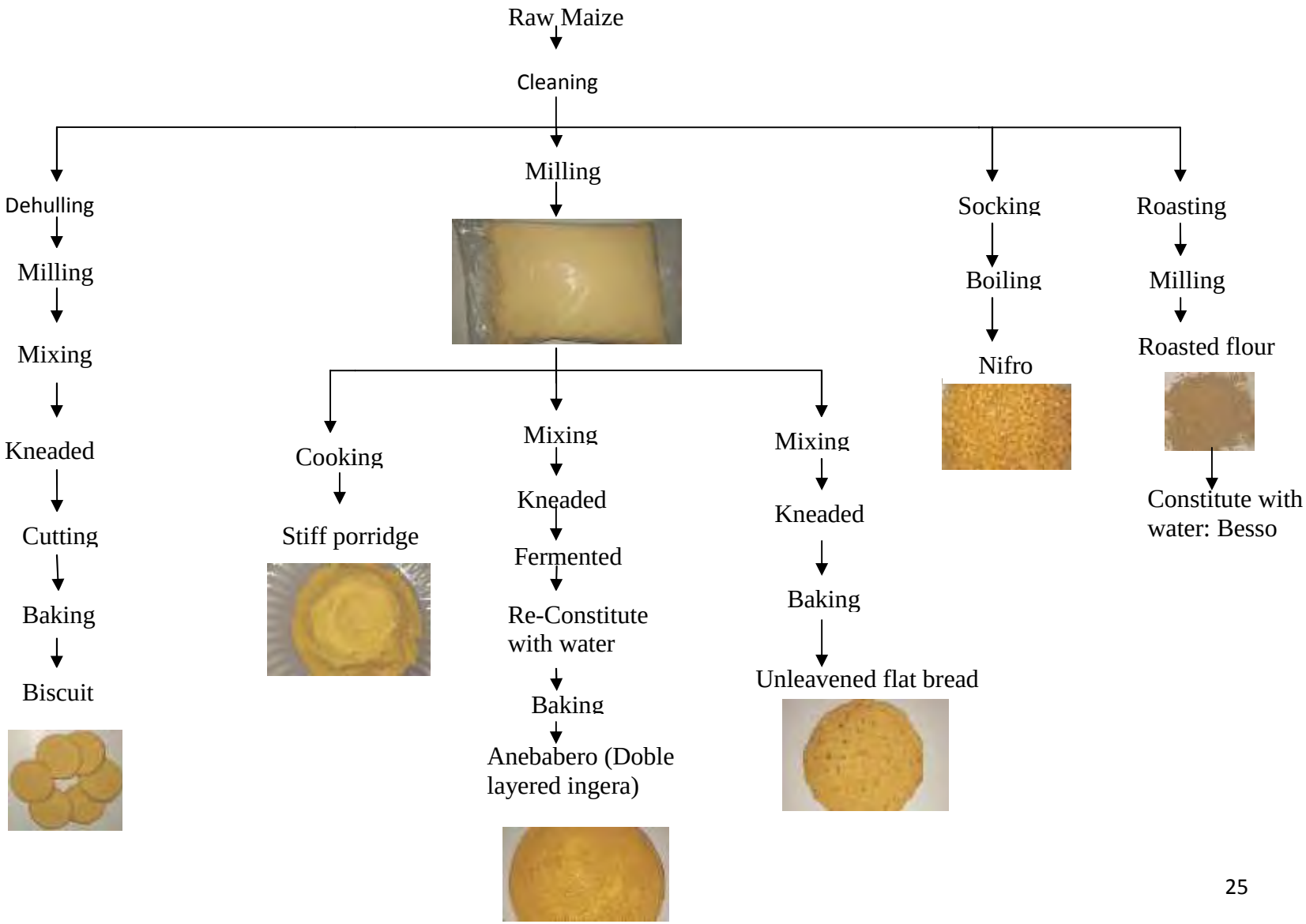


Figure 7: Food preparation flow diagram

Remark: Melkassa 1 β-carotene content was higher than other varieties 3.1±0.1. Due to that it was selected for retention study

4.3 Preparation of Biscuit

- Preparation of Maize Flour

The method of (Houssou and Ayemor 2002 sited by Bibiana et al 2014) was used to prepare the maize flour. Maize kernels were sorted to remove stones, dirt and other foreign materials. Water was sprinkled on cleaned maize seeds so as to allow absorption of water by the grains, toughening the pericarp and germ. The grains were left for about 10 min before dehulling and milling. The flour was sieved using 250 μm mesh size.

- Biscuit Processing

Flour and baking powder, was mixed by hand. Water was added gradually and the mixture was kneaded for 3 min at medium speed in an electric mixer to firm dough. The dough was manually rolled out on a steel tray, to a height of 5 mm and cut into circular shapes using a 6.3 cm diameter biscuit cutter. The dough pieces were transferred onto a baking tray lined with aluminium foil. The biscuits was baked in a preheated electric oven at 200^0C for 30 min and cooled for 30 min at ambient temperature. Biscuits were packed in polyethylene bags and stored at 4^0C until subsequent carotinoid analysis (Serrem, 2011).

4.4 Chemicals and Standards

All solvents used in the analysis of carotenoid were HPLC grade. The following solvent were used acetone, petroleum ether, acetonitrile, methanol, ethyl acetate, triethylamine, DMSO (Dimithyl Sulfoxide). An analytical standard of β – carotene (Sigma-Aldrich, St. Louis, MO, USA) was used to calibrate and quantify the β- carotene. All chemicals and reagents used for laboratory analysis of other parameters were analytical grade.

4.5 Method of Analysis

4.5.1 Sample Collection and Sample Preparation

Ten kg of maize was taken from each variety from Ambo Agricultural Research Centers, Melkasa Agricultural Research Centers and Ethiopian Seed enterprise (Bahir Dar and

Gibie Awash branch) to the laboratory. The grains (approximately 1 kg) were evenly piled on a clean surface; the pile was flattened and spread into a circle. A cross was made and dividing the circle into four roughly equal parts. The two opposite quarters was diametrically removed and the other was remixed. The quartering procedure was repeated until the amount was reduced to approximately 250 g (Rodriguez-Amaya & Kimura 2004). The dried seeds were ground in grind mill (FW100). The finely grinded flour was sieved using 500µm sieve and the powder was kept in amber glass vials sealed with a lid, wrapped in aluminum foil. (Rodriguez-Amaya & Kimura 2004; Rios et al., 2014). Samples were kept in refrigerator (4°C) until they were needed for analysis. They were brought up to 25°C before analysis (Dixon 2000).

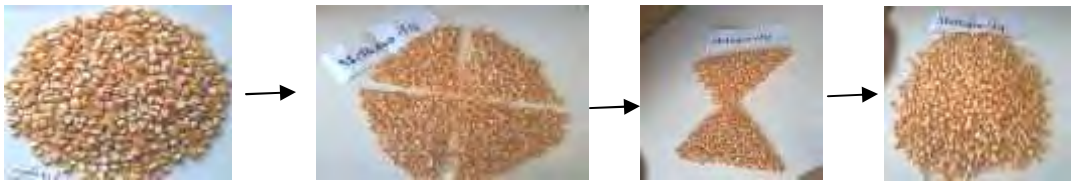


Figure 8: Illustration of quartering of grains and flours. Source: Rodriguez-Amaya & Kimura (2004).

Quartering was performed, as described above for cooked foods as well. Wet prepared food from maize samples were dried by using freezes dryer to reduce the moisture content of the food sample. Each well dried food sample was grinded to a fine smooth-texture sample with the aid of coffee grinder in order to increase the surface area of the food sample for the subsequent analysis (Sanusi and Adebiyi, 2009).

4.5.2 Total Caroteniod and Beta Carotene Analysis

4.5.2.1 Beta Carotene Standard Preparation

The standard was prepared by using crystal form of 95% HPLC grade beta carotene type II produced by Sigma Aldrich. Stock solution was prepared 30µg/ml in DMSO (Dimithyl Sulfoxide).

4.5.2.2 Calibration for Beta Carotene Analysis

All calibration points were prepared from the stock standard solution through a serial dilution to be 6 to 0.1µg/ml in acetone. A 6 point calibration curve was plotted from 0.1 to 6.0 µg/ml. The calibration curve was linear (r^2=0.99770).

In addition, absorbance accuracy of UV Spectrophotometer for total Carotenoid Analysis was checked according to the procedure of the Association of Official Analytical Chemists (AOAC 1997, as cited in Rodriguez-Amaya & Kimura 2004), Solution of 0.0400 g K_2CrO_4 per liter of 0.05 N KOH was prepared and the absorbance was measured. 0.05 N KOH solution was used as blank. Each wave length absorbance was check two times. Expected absorbance and the mean of the two actual absorbance of each wave length were statistically compared using one sample t-test at 95% degree of confidence. The expected and actual absorbance at specified wavelengths was shown below.

Table 5: Absorbance accuracy of UV spectrophotometer for total carotenoid analysis

Wavelength (nm)	Expected Absorbance	Actual Absorbance
230	0.171	0.16828
275	0.757	0.78152
313.2	0.043	0.04149
375	0.991	1.01300
400	0.396	0.40317

4.5.2.3 Extraction and Partition of Carotenoid

The analysis was done based on the method described by Rodriguez-Amaya & Kimura (2004) under low light conditions. Extraction and partition were carried out under a hood.

Dry corn is difficult to extract. Rehydration allows efficient penetration of the extraction solvent into the corn tissues. Acetone is used in this method because it is inexpensive and

readily available and it penetrates food tissue well. 0.1% BHT was added as antioxidant to all solvents. 3g of ground corn was taken and sufficient deionized water (about 10ml) was added to cover the surface, then allowed to stand for 30 minutes. 20ml of cold acetone was added and left to stand for 15 minutes. Then the solution was filtered using whatmann No. 4 on a Buchner funnel. The solid was placed in a mortar, ground well with the pestle, about 50ml of cold acetone (acetone refrigerated for about 2 hours) was added, and that was grounded again with the pestle to extract the carotenoids. It was filtered through the same funnel and collected in the same flask. The mortar, pestle, funnel, and residue were rinsed with small amounts of acetone; the rinse was received in the flask with extract. The extraction was repeated until the residue become colorless.

The extract was partitioned between petroleum ether (PE) and water in a separator funnel. 20ml of petroleum ether was placed in a separator funnel then one third of the extract and 300 ml of deionized water were added. The deionized water was added slowly down the wall of the separator funnel to minimize emulsion. The aqueous lower phase was discarded. The partitioning was repeated for the rest portions of the extract sequentially. After the third portion was portioned the PE layer was washed three times with deionized water to remove acetone. In the last washing, it was discarded the lower phase as completely as possible without discarding any of the upper phase.

The upper phase was collected by passing it through a small funnel with anhydrous sodium sulphate (~15g) to remove residual water in 25ml volumetric flask for total carotenoid analysis and in 50ml round bottom flask for β-carotene analysis. Glass wool was used to plug the funnel to hold the sodium sulfate. The funnel was washed with small amount of petroleum ether collecting the washing into the flasks.

4.5.2.4 Measuring of Total Carotenoid Level

The 25ml volumetric flask was made up to the mark with petroleum ether and it was used to measure the total carotenoid at 450nm by using Agilent 8653 UV-Visible spectrophotometer. The total carotenoid content was calculated using the formula:

Total carotenoid content (µg/g) = $\frac{A_{Total} \text{ x volume (ml) x } 10^4}{A^{1\%}_{1cm} \text{ x sample weight (g)}}$

Where Atotal= absorbance; volume = total volume of extract (25 ml); A1% 1cm = absorption coefficient of 2500, which is recommended for mixtures.

4.5.2.5 HPLC Analysis of β-Carotene

The collected extract in 50ml round bottom flask from separatory funnel was concentrated in a rotary evaporator T $\leq$ 35^0c and dried under nitrogen gas then that was redissolved by using 1ml of HPLC grade acetone. Before injection to HPLC, it was filtered through 0.22 µm PTFE syringe filter (millipor) directly into sample vials.

β-carotene analysis was performed using Agilent 1260 infinity series consist of quaternary pump, autosampler, column thermostat and chemstation software. The carotenoids were separated on a Monomeric C18 column: Waters Spherisorb ODS 2 (5µm, 4.6 x 250mm) operated at a flow rate of 0.5ml/min. Three mobile phases were used and they were mixture of acetonitrile, methanol, ethyl acetate with 0.05% triethylamine. The gradient elution program was set as follows 95:5:0 to 60:20:20 in 20 min, staying in this proportion until 40 min, then to 20:40:40 in 60 min to remove the lipids re-equilibration was for 15 minute. The column temperature was 30^oc and the wave length of UV/visible was 450nm. The injection volume was 10µl. The beta carotene content was calculated using the formula:

C_x (µg/g)= $\frac{A_x \text{ x } C_s \text{ (µg/ml)x total volume of extract (ml)}}{A_s \text{ x sample weight (g)}}$

Where: C_x is concentration of carotenoid of sample

A_x is peak area of carotenoid of sample

C_s is concentration of the standard

A_s is peak area of the standard.

- **Retention**

  Retention was calculated by using apparent retention. Apparent retention is defined as the ratio of the nutrient content in the cooked food to the nutrient content in the raw food, expressed on a dry weight basis (Murphy et al., 1975 as sited in Rodriguez-Amaya & Kimura 2004; Li et al., 2007), which is as follows:

$$\%\text{Apparent retention} = \frac{\text{Nutrient content per g of cooked food (dry basis)}}{\text{Nutrient content per g of raw food (dry basis)}} \times 100$$

4.5.2.6 Recovery Experiment for β-carotene Analysis

The recovery was used to determine the method accuracy. Accuracy is the degree of agreement of a measurement or average of measurements with an accepted reference or 'true value', and is a measure of bias in the system. In this study the accuracy of the technique was evaluated in terms of % recovery. For the analysis of the recovery values between 70% and 120% were considered as acceptable range.

$$\%R = \frac{S-U}{C} \times 100$$

Where: % R is Percentage recovery

S is measured concentration in spiked sample

U is measured concentration in unspiked sample

C is actual concentration added to the sample

The recovery was performed by spiking sample with known amount of beta carotene standard (1µg/ml) and analyzed as per the method and the results were compared to determine the effect the matrix on the accuracy of the analysis. The method recovery was 76.4%.

4.5.2.7 Analysis Repeatability

Repeatability, expressed as the relative standard deviation (RSD), consists of multiple measurements of a sample by the same analyst under the same analytical conditions. In this study the repeatability of analytical method was assessed by analyzing six replicate samples within a day. Relative standard deviation of six analyses of a sample was 3.4%.

Relative standard deviation (RSD) was calculated as follows:

$$RSD = \frac{S}{X} \times 100$$

Where: S= standard deviation of replicate analyses

X= mean of the replicate analyses

4.5.2.8 Limit of Detection

Detection is the lowest concentration of analyte in sample that can be detected, not necessarily quantitated under stated experimental conditions. The limit of detection was defined as the concentration value of the studied compound for which the signal (S) to noise (N) ratio was higher than 3 (S/N>3) (FDA, 1994). In this study, the instrument detection limit was performed by preparing serial dilution of β-carotene standard for 0.07µg/ml, 0.06µg/ml and 0.05µg/ml and run each of them seven times. The signal (S) to noise (N) ratio higher than 3 (S/N>3) was obtained for 0.07µg/ml.

4.5.2.9 Quality Control

All glassware and equipments such as analytical balance that used during total carotenoid and β-carotene analysis were calibrated by National Metrology Institute of Ethiopia, and intermediate check was done before use each time. Method blank was run before a batch of analyzed sample and analysis every day; replicate analysis, spiked sample were used in every batch of sample analysis.

4.5.3 Proximate Composition Determination

4.5.3.1 Moisture Content

The moisture content of the sample was determined according to (AOAC, 2005). About 10.0 g weight of sample was taken into a previously dried (at 105^0C) moisture analysis dish and the weight of the sample was determined as M1. The sample was dried at 105^0c for 24 hours. and cooled in desiccators for about 30 minutes and weighed accurately and recorded as M2. The moisture content was expressed as percentage of the dry weight.

$$\text{Moisture content} = \frac{M2}{M1} \times 100$$

Where:

M1=Weight before drying

M2= Weight after drying

4.5.3.2 Determination of Total Ash

The ash content was determined by gravimetric method (AOAC, 2005). A cleaned crucible was placed in a furnace and ignited at 550^0C for 1 hour. 2.5g of the dried sample was weighed in to a dry porcelain dish and then heated in a muffle furnace at 550^0C for 6 hours. It was cooled in desiccators and weighed. The percentage ash content was calculated as follows:

$$\% \text{ ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

4.5.3.3 Determination of Crude Protein

Protein (N×6.25) was determined by Kjeldahl method. In this method, proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. Then, the total organic nitrogen was converted to ammonium sulfate. The digest was neutralized with alkali and distilled into a boric acid solution. The borate anions formed were titrated with standardized HCl, and were converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food (AOAC, 2000).

Addition of reagent: 0.5 g of sample was weighed in a tecator tube and placed in the tecator rack. Then 6 ml of sulfuric acid mixture was added carefully by using a pipette and mixed with the sample immediately. Then 3.5 ml of 30% hydrogen peroxide was added step by step till the reaction stops. As soon as the reaction has ceased, the tube was hand-shaken for a few minutes and was put back into the rack. Then, 3 g of catalyst (K_2SO4 mixed with copper sulphate) was added and the mixture was allowed to stand for 5-15 minute before digestion.

Digestion: The tube in the rack was lowered into the digester in the fume hood at $370^{O}C$. The exhaust manifold was located on top of tubes and the digestion was continued until clear solution appeared 3-4 hours in the fume hood. After digestion was completed, the content in the flask was diluted by water and concentrated sodium hydroxide (40%) was added to neutralize the acid and to make the solution slightly alkaline.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

The ammonia was then distilled into a receiving flask that consisted solution of excess boric acid. The borate ion was formed as a result of the reaction of the boric acid and the ammonia and this was titrated with standard acid (0.1HCl) until the green color changes to pink.

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

$$\text{Total nitrogen (\%)} = \frac{[(V-V\mathbf{b}) \times N \times 14)]}{W}$$

Crude protein (%) = total nitrogen (%) × 6.25

Where: V = volume of hydrochloric acid consumed to neutralized the sample

Vb= the volume of hydrochloric acid consumed to neutralize the blank

N × 14 = normality of the acid × equivalent weight of nitrogen

6.25 = conversion factor from total nitrogen to crude protein

W = weight of sample on dry basis

4.5.3.4 Crude Fat Analysis

Fat was determined by exhaustively extracting sample in petroleum ether (boiling point, 40 to 60^{O}C) in soxhlet extractor (AOAC, 2005). A 2.00g (M) of the sample was weighed into an extraction thimble and covered with absorbent cotton. Then 50 ml solvent (petroleum ether) was added to a pre-weighed cup (M_1). Both the thimble and the cup would be attached to the extraction unit. The extraction process continue for 4 hours and then this flask with its content was removed from the soxhelet and placed into drying oven at 92^{O}C for 30minutes, and placed into desiccators for 30 minutes. Finally the mass of each flask together with its fat contents was measured as (M_2). Then the total content of the fat was calculated using the following formula

$$\text{Crude Fat \%} = \frac{M_2 - M_1}{M} X100$$

Where: -M_2 is mass of flask and lipid extracted

M_1 is mass of dried flask

M is weight of samples on dry basis

4.5.3.5 Crude Fiber Analysis

Crude fiber was determined as per ES ISO 5498:2002. About 1 g of sample was weighed into a 600 ml beaker (W1). 200ml of 1.25% sulphuric acid solution was added at room temperature and boiled for 30 minutes. The beaker was swirled to mix any particles adhering to the interior wall of the beaker.20ml of 28% potassium hydroxide solution was added and boiled for 30 minute, stirred occasionally. Sintered glass crucible was covered with 10mm sea sand and the layer of sand was wetted by distilled water. Then the mixture was filtered through sintered glass crucible with a layer of sea sand using vacuum pump. The beaker was washed repeatedly with hot distilled water until the

residue removed and transferred it to crucible. The residue was washed three times under vacuum each time with 30ml of 1% sulphuric acid solution and then distilled water, followed by 1% sodium hydroxide solution and then distilled water and acetone. After washing, the residue was dried by suction. The residue was dried at 130^0c for 2 hours in dry air oven. The crucibles with residue were cooled in the desiccator and weighed (W2). Then the residue incinerated at 550^0c for 2 hours in muffle furnace. After that the crucibles with residue were cooled in desiccator and weighed (W3). The crude fiber content was calculated by using the following formula.

$$\% \text{ Crude fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

Where: W_1 is weight of sample (g)

W_2 is weight of crucible and residue after drying (g)

W_3 is weight of crucible and residue after incineration (g)

4.5.3.6 Total Carbohydrate

The total carbohydrate was determined by differential method (Enyisi et al., 2014). This was achieved by subtracting the total protein, lipid, moisture and ash content from 100 thus: % carbohydrate= (100 – (% moisture + % ash+ % fat + % protein + %fiber).

The gross energy of each sample was estimated (in kcal/g) by multiplying the percentages of crude protein, crude fat and carbohydrate with recommended factors.

Total energy (in kcal/100g) = (9*crude fat + 4*crude protein + 4* carbohydrate)

4.5.3.7 Analysis of Mineral

Mineral was determined as per AOAC 2000. Ash was obtained from dry ashing of samples. The ash was dissolved by 7ml of 6N HCl and carefully dried at low temperature on hot-plate. 15ml of 3N HCl was added and the crucibles were heated on the hot plate until the solution just boiled. The solution was cooled and filtered through a filter paper (Whatman 42, 125mm) in to 50ml volumetric flask. Again 10ml of 3N HCl was added to

the crucible to dissolve the residue and it was heated on a hot plate until the solution just boiled. Then the solution was cooled and filtered into the previous 50ml volumetric flask. The crucible was washed and filtered and combined the washing into 50ml volumetric flask .Then 5 ml of 10% lanthanum chloride solution was added in 50ml volumetric flask and the flask filled with distilled water up to the mark. The blank was prepared by taking the same amount of reagents through the steps all of the above without the sample. The instrument was set and optimized based on the instruction given in the manual. The calibration solutions and the reagent blank solutions were measured first. Then the samples were run following the calibration values. The calibration curve was prepared for the required metal by plotting the absorption values against the metal concentration in ppm. The sample concentration was analyzed using AAS (Varian SpectrAA-20 Plus).

Table 6: Series of working standard solution for determination of minerals

Series no.	Standard concentration(ppm)
1	0
2	1
3	2
4	3
5	4

$$\text{Metal Content (mg/100g)} = \frac{(a-b) \times v}{10 \times w}$$

W = Weight of the sample in gram

a = concentration in ppm of sample solution

v = volume of the extract (50ml)

b = concentration in ppm of blank solution

4.5.4 Determination of Phytate

Phytate was determined by following AOAC 2000. About 0.2 g of dried sample was extracted with 10 ml of 0.2N HCl for 1 hour at an ambient temperature. The extracted sample was centrifuged for 30 minutes at 3000 rpm and the clear supernatant was used for phytate determination. 2 ml of wade reagent was added to 3 ml of supernatant sample solution that was homogenized and centrifuged for 10 min at 3000 rpm. Wade reagent was prepared by mixing equal amount volume of 0.03% $FeCl_3.6H_2O$ and 0.3% Sulfosalcilic acid. The absorbance of the solution was measured at 500 nm using UV-Vis spectrophotometer. The phytate content was calculated from the difference between the absorbance of the blank (3 ml of 0.2N HCl + 2 ml of wade reagent) and that of assayed sample. The amount of phytic acid was calculated using phytic acid standard curve and the result was expressed as phytic acid in mg/100g DW.

Standard solution preparation: A series of standard solution was prepared containing 0, 4, 9, 18, 27, 36 µg/g .Phytic acid in 0.2N HCl. Then, 3 ml of each standard was pipette into 15 ml centrifuge tubes and 3 ml 0.2N HCl (blank). Then, 2 ml of wade reagent was added to each tube, and the solution was mixed on a vortex mixer for 5 second. The mixture was then centrifuged for 10min at 3000 rpm and the supernatant read at 500 nm. The calibration curves (absorbance vs. concentration) and the slope and intercept were calculated as follows:

$$\text{Phytic acid in µg/g} = \frac{[(Ab-As)-\text{Intercept}] \times 10}{\text{Slope} \times w \times 3}$$

Where:-As is Absorbance of the test sample,

Ab is Absorbance of the blank

W is Weight of sample

Determination of molar ratio for phytate/mineral:- the mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytae:660g/mol; Zn:65g/mol; Fe:56g/mol). The molar ratio between phytate and

mineral was obtained after dividing the mole of phytate with the mole of minerals. Foods phytate to zinc ratio >15 and phytate to iron ratio >1 had poor bioavailability of zinc and iron (Norhaizan & Nor, 2009).

4.6 Sensory Evaluation

Taste, Color, texture, aroma and over all acceptability were assessed by Food Science and Nutrition students at the center. For this purpose 33 persons were selected randomly. The samples were given three digits codes to prevent bias. The method was 7 points hedonic scale 7 indicated extremely like and 1 indicated extremely dislike. The food with high retention of β-carotene was selected for sensory analysis.

4.7 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 16.0 was used to analyze the data. Descriptive statistic mean, standard deviation (SD) and range were calculated from analysis and the data was expressed as mean $\pm$ SD. The mean were statistically compared by using one way ANOVA, student t-test and LSD. Differences in means were considered significant at level of $P < 0.05$. All data were expressed in dry weight (DW).

5 RESULTS

5.1 Total Carotenoids and β-carotene in Maize Varieties

The total carotenoid and beta carotene of maize varieties are presented in Table 7. The total carotinoid content in the yellow maize varieties ranged from 11.4 ± 0.2 for Melkassa 7 to 28.9 ± 0.3 µg/g for Melkassa 1, with β-carotene ranging from 1.2± 0.1 for Gibie Awash to 3.1 ± 0.1 µg/g for Melkasa 1.However, carotenoids were below detection limit in the white maize variety (BH 660). Statistically there was significant difference ($P < 0.05$) among yellow maize varieties in total carotenoid and in beta carotene content except between melkasa 1 and melkassa 1q ($P > 0.05$).

Table 7: Beta carotene and total carotenoid composition of maize varieties

Maize Varieties	Kernel Colour	β carotene µg/g (DW)	Total Carotenoid µg/g (DW)
CML 161	Yellow	ǂ 2.2 ± 0.1^{c}	22.6±0.4^{d}
Mellkassa-1q	Yellow	3.0 ± 0.1^{a}	27.6±0.2^{b}
Mellkassa-1	Yellow	3.1± 0.1^{a}	28.7±0.3^{a}
BHQMY 545	Yellow	2.7±0.1^{b}	25.6±0.3^{c}
CML 165	Yellow	1.7 ± 0.1^{d}	19.0±0.2^{e}
Mellkassa-7	Yellow	1.5± 0.2^{e}	11.4±0.2^{g}
Gibie Awash	Yellow	1.2± 0.1^{f}	17.0± 0.2^{f}
BH 660	White	ND	ND

Values within the same column followed by different superscripts are significantly different ($P<0.05$)

ǂ such values are the mean ± SD of triplicate;

ND is not detected.

5.2 Retention of Beta Carotene in Prepared Foods from Melkasa 1

The maize variety Melkasa 1 with beta carotene content 3.1± 0.1 was used to prepare traditional foods. The level of β-carotene in traditionally prepared foods and their retention are revealed in Table 8. β- carotene ranged 1.7±0.1 µg/g for besso to 2.8±0.1 µg/g for stiff porridge. There was significant difference ($p<0.05$) in the mean retention of beta carotene among processed foods except between stiff porridge and Unleavened flat bread ($p<0.05$).

Table 8: β-carotene content of prepared foods from Melkasa 1 and their retention

Foods	β carotene µg/g (DW)	Retention %	µgRE/100g
Anebabero	ǂ 2.5±0.1[b]	80.5[b]	125
Stiff porridge	2.8±0.1[a]	88.9[a]	140
Unleavened flat bread	2.7±0.1[a]	88.3[a]	135
Biscut	2.1±0.1[d]	67.1[d]	105
Besso	1.7 ±0.1[e]	55.9[e]	85
Nifro	2.4±0.1[c]	75.8[c]	120

Values within the same column followed by different superscripts are significantly different ($P<0.05$). Beta carotene is converted to RE as per the conversion factor used by Ejigui et al (2005) to convert beta carotene to RE in yellow maize (1RE=3.33 International Unit (IU); 1IU= 0.60 µg β carotene; 1RE= 3.33x0.6µg β-carotene approximately 2 µg β-carotene).

ǂ such values are the mean ± SD of triplicate.

Table 9: Percentage provide for daily mean requirement intake for different age and gender group from 100g yellow maize foods

Group	Age	Mean requirement µg RE/day	Anebabero 125 µg RE/100g	Stiff porridge 140 RE/100g	Unleavened flat bread 135 RE/100g	Biscut 105 RE/100g	Besso 85 RE/100g	Nifro 120 RE/100g
Infants and children	0-6 months	180	69.4	77.8	75.0	58.3	47.2	66.7
	7-12 months	190	65.8	73.7	71.1	55.3	44.7	63.2
	1-3 years	200	62.5	70.0	67.5	52.5	42.5	60.0
	4-6 years	200	62.5	70.0	67.5	52.5	42.5	60.0
	7-6 years	250	50.0	56.0	54.0	42.0	34.0	48.0
Male	12-Nov	500	25.0	28.0	27.0	21.0	17.0	24.0
	13-15	600	20.8	23.3	22.5	17.5	14.2	20.0
	Adult (15)	600	20.8	23.3	22.5	17.5	14.2	20.0
Adolescents	10-18 years	365	34.2	38.4	37.0	28.8	23.3	32.9
Adults: Female	19-65 years	270	46.3	51.9	50.0	38.9	31.5	44.4
	65+years	300	41.7	46.7	45.0	35.0	28.3	40.0
Male	19-65 years	300	41.7	46.7	45.0	35.0	28.3	40.0
	65+years	300	41.7	46.7	45.0	35.0	28.3	40.0
Pregnant women	-	370	33.8	37.8	36.5	28.4	23.0	32.4
Lactating women	-	450	27.6			23.3	18.9	26.7

5.3 Proximate Composition and Mineral Content of Maize Varieties

5.3.1 Proximate Composition

The proximate composition of maize verities is presented in Table 10. The mean values obtained for moisture contents of maize varieties ranged 9.4 % for Melkasa 1q to 11.5% for BH 660. There was a significant difference ($p<0.05$) in moisture content among maize varieties except between CML 165 and Melkassa-7 and between Melkassa 1 and BHQMY 545. All the mean values of moisture content of the maize varieties fall within the acceptable limit of moisture content of maize that is less than or equal to 13% (ES 679:2001).

The maize varieties total ash content ranged 1.4% for BH 660 to 1.7% for BHQMY 545. There was significant difference ($P<0.05$) among maize varieties in total ash content. Crude protein content of maize varieties ranged 9.6% for CML 165 to15.7% for BHQMY 545. There was significant difference ($P<0.05$) among maize varieties in crude protein content except between Melkasa-1 and BH 660 ($P>0.05$).

The maize varieties crude fat content ranged from 5.0% for Melkasa-7 to 7.2% for Melkasa-1q. Crude Fat content in maize varieties was significantly different ($P<0.05$) except between Melkasa-1 and Gibie Awash. Crude fiber content of maize varieties ranged from 1.6% for Melkasa 1q to 3.5% for BHQMY 545. There was significant difference ($P<0.05$) among maize varieties

Percent carbohydrate content of maize varieties was found in the range of 62.2% for BHQMY 545 to 70.0% for CML 165. There was significant difference ($P<0.05$) among maize varieties. Energy content of maize varieties was found in the range of 367.1 kal/100g for CML 161 to 387.1 kal/100g for CML 165. There was significant difference ($P<0.05$) among maize varieties in energy content.

Table 10: Proximate values in maize varieties

Varieties	Moisture (%)	Total Ash (% DW)	Crude Protein (% DW)	Crude Fat (% DW)	Crude Fiber (% DW)	Carbohydrate (% DW)	Energy kal/100g (DW)
CML 161	ǂ 11.3 ± 0.0^{b}	1.4±0.0^{e}	12.0±0.1^{d}	5.1±0.0^{d}	2.2±0.0^{c}	67.7±0.3^{c}	367.2±1.9^{c}
Melkasa-1q	9.4 ±0.0^{g}	1.5±0.0^{d}	13.1±0.1^{b}	7.2±0.0^{a}	1.6±0.0^{e}	66.9±0.6^{c}	387.1±1.8^{a}
Melkasa-1	10.5± 0.0^{d}	1.6±0.0^{b}	12.5±0.1^{c}	6.2±0.0^{c}	1.7±0.0^{de}	67.6±0.1^{c}	375.7±0.2^{b}
BHQMY 545	10.6±0.0^{d}	1.7±0.0^{a}	15.9±0.0^{a}	6.7±0.0^{b}	3.5±0.1^{a}	62.2±0.5^{d}	375.6±2.1^{b}
CML 165	9.9 +0.0^{e}	1.6±0.0^{cd}	9.7±0.1^{f}	6.1±0.0^{c}	2.6±0.1^{b}	70.0±0.3^{a}	370.6±2.6^{c}
Melkassa-7	9.9 ±0.0^{e}	1.6±0.0^{c}	11.9±0.1^{d}	5.0±0.0^{d}	2.5±0.2^{b}	69.0±0.1^{b}	370.3±1.8^{c}
Gibie Awash	11.0±0.0^{c}	1.5±0.0^{d}	10.3 ±0.2^{e}	6.1 ±0.0^{c}	2.3 ±0.0^{c}	69.0 ±0.3^{b}	369.3±1.6^{c}
BH 660	11.5±0.0^{a}	1.4±0.0^{f}	12.3±0.1^{c}	5.3±0.0^{d}	1.8±0.0^{d}	67.7±0.5^{c}	368.4±2.0^{c}

Values within the same column followed by different superscripts are significantly different (P<0.05).

ǂ such values are the mean ± SD.

5.3.2 Mineral (Fe, Zn and Ca) Content of Maize Varieties

Data regarding the three minerals content of maize varieties is given in Table 11. The result showed for the three mineral content ranged 2.3mg to 3.7 mg for Fe, 2.3mg to 2.9mg for Zn and 29.3mg to 48.1mg for Ca. There was significant difference ($P<0.05$) among maize varieties in the three mineral content.

Table 11: Fe, Zn and Ca of maize varieties (mg/100g)

Varieties	Kernel color	Fe (mg/100g DW)	Zn (mg/100g DW)	Ca (mg/100g DW)
CML 161	Yellow	ǂ 3.7±0.1^{a}	2.5±0.1^{b}	34.4±0.0^{d}
Mellkassa-1q	Yellow	2.3±0.0^{f}	2.5±0.0^{b}	30.9±0.1^{f}
Mellkassa-1	Yellow	2.9±0.0^{d}	2.5±0.0^{b}	29.3±0.0^{g}
BHQMY 545	Yellow	2.5±0.0^{e}	2.5±0.0^{b}	34.9±0.0^{c}
CML 165	Yellow	3.7±0.0^{a}	2.3±0.1^{c}	22.0±0.1^{h}
Mellkassa-7	Yellow	3.3±0.1^{b}	2.9±0.0^{a}	36.3±0.1^{b}
Gibie Awash	Yellow	3.2±0.1^{c}	2.9±0.0^{a}	48.1±0.1^{a}
BH 660	White	2.4±0.0^{f}	2.5±0.1^{b}	31.3±0.1^{e}

Values within the same column followed by different superscripts are significantly different ($P<0.05$).

ǂ such values are the mean ± SD.

5.4 Phytate in Maize Varieties

The result of phytate content is presented in Table 12. Phytate content of maize varieties were found in the range of 71.7mg for Melkasa 7 to 146.2 mg for BHQMY 545. Phytate to zinc molar ratio 2.5 for Melkasa 7 to 5.8 for BHQMY 545 and phytate to iron molar ratio1.8 for CML 165 to 5.1 for BHQMY 545. There was significant difference ($P<0.05$)

among maize varieties in phytate content, phytate to zinc molar ratio and phytate to iron molar ratio.

Table 12: Phytate and bioavailability estimates in maize varieties

Varieties	Kernel color	Phytate mg/100g	Phy/Zn molar ratio	Phy/Fe molar ratio
CML 161	Yellow	ǂ 88.8±0.2^{e}	3.5±0.1^{d}	2.0±0.1^{e}
Melkassa-1q	Yellow	78.6±0.2^{f}	3.2±0.0^{e}	2.9±0.0^{d}
Melkassa-1	Yellow	121.2±0.2^{b}	4.7±0.0^{a}	3.6±0.0^{b}
BHQMY 545	Yellow	146.2±0.2^{a}	5.8±0.0^{b}	5.1±0.0^{a}
CML 165	Yellow	79.1±0.2^{f}	3.4±0.1^{d}	1.8±0.0^{h}
Mellkassa-7	Yellow	74.8±0.2^{h}	2.5±0.0^{f}	1.9±0.1^{f}
Gibie Awash	Yellow	106.7±0.2^{c}	3.6±0.0^{c}	2.9±0.1^{d}
BH 660	White	95.2±0.2^{d}	3.7±0.1^{d}	3.3±0.0^{c}

Values within the same column followed by different superscripts are significantly different ($P<0.05$).

ǂ such values are the mean ± SD.

5.5 Proximate and Mineral Contents in Maize Foods Prepared from Melkasa 1

5.5.1 Proximate Composition

The proximate composition of foods and raw maize (melkasa 1) is shown in Table 13. Moisture content of foods ranged from 5.4% for besso to 10.2% for biscut. Percent crude fat obtained for foods ranged 5.0% for biscuit to 5.7% for unleavened flat bread. The crude fat content of foods was significantly different ($P<0.05$) from the crude fat content of raw maize (melkasa 1). Fat content of stiff porridge, unleavened flat bread, nifro, anebabero, biscut and besso reduced by 9.8%, 6.6%, 9.8 %, 9.8%, 18.0% and 8.2% respectively. The loss of fat among foods was significant different ($P<0.05$).

The crude protein content of prepared foods ranged 10.6% for nifro to 11.9% for unleavened flat bread. The loss of crude protein in the foods stiff porridge, unleavened flat bread, nifro, anebabero, biscut and besso during preparation was 5.6%, 4.6%, 14.8 %, 9.7%, 9.7% and 7.3% respectively. There was significant difference ($P<0.05$) between foods and raw maize in protein content.

The total ash content of the prepared foods ranged from 1.3% to 1.5%. The total ash content of foods exhibited significant difference ($P<0.05$) when compared to raw maize (Melkasa 1). There was significant difference ($P<0.05$) among prepared foods in total ash content. It reduced in prepared foods by 6.3% (stiff porridge), 6.3% (unleavened flat bread), 18.8 % (nifro), 12.5 (anebabero), 12.5% (biscuit) and 6.3% (besso).

Crude fiber content of prepared foods ranged from 1.3% biscuit to 1.5% nifro. There was significant difference ($P<0.05$) between raw maize (1.7%) and processed foods while there was no significant difference ($P>0.05$) among processed foods except biscuit. Crude fiber during cooking reduced by 8.9% (stiff porridge), 8.9% (unleavened flat bread), 6.5% (nifro), 8.9% (anebabero), 21.9% (biscuit) and 6.5% (besso). 86.7% of maize kernel crude fiber is found in pericarp (Hoopen and Maiga, 2012), dehulling may be one of the reasons for high reduction of crude fiber in biscuit.

There was significant difference ($P<0.05$) between processed foods and raw maize (melkasa 1) in total carbohydrate and energy content. All processed foods show higher energy and carbohydrate content than raw maize except biscuit in energy. The carbohydrate was calculated by difference and the reduction of other nutrients during processing may be the reason for increasing of carbohydrate and energy in processed foods.

Table 13: Proximate composition of raw maize (Melkase 1) and prepared foods

Sample Type	Moisture %	Total Ash %	Crude Protein %	Crude Fat %	Crude fiber %	Carbohyd rate %	Energy (kcal/100g)
Melkasa 1	ǂ 10.5±0.0^{a}	1.6±0.0^{a}	12.5±0.1^{a}	6.1±0.0^{a}	1.7±0.0^{a}	67.6±0.1^{d}	375.7±0.2^{f}
stiff porridge	9.4 ± 0.0^{c}	1.5±0.0^{c}	11.8±0.0^{bc}	5.5±0.0^{c}	1.5±0.0^{b}	70.3±0.0^{c}	378.1±0.1^{e}
Unleavened flat bread	9.0 ±0.0^{d}	1.5±0.0^{c}	11.9±0.2^{b}	5.7±0.0^{b}	1.5±0.1^{b}	70.4±0.0^{c}	380.6±0.3^{c}
Nifro	9.0± 0.0^{d}	1.3±0.0^{e}	10.6±0.3^{e}	5.5±0.0^{c}	1.6±0.0^{b}	71.9±0.3^{b}	379.7±0.0^{d}
Anebabero	8.2 ±0.0^{d}	1.4±0.0^{d}	11.3±0.2^{cd}	5.5±0.0^{c}	1.5±0.0^{b}	72.2±0.3^{b}	382.9±0.0^{b}
Biscut	10.2 ±0.0^{b}	1.4±0.0^{d}	11.3±0.1^{d}	5.0±0.0^{d}	1.3±0.1^{c}	70.8±0.1^{c}	373.1±0.3^{g}
Beso	5.4 ±0.0^{f}	1.5±0.0^{c}	11.6±0.2^{bcd}	5.6±0.0^{b}	1.6±0.0^{b}	74.5±0.2^{a}	394.0±0.1^{a}

Values within the same column followed by different superscripts are significantly different (P<0.05).

ǂ such values are the mean ± SD.

5.5.2 Mineral Composition in Foods Prepared from Melkasa 1

Result of foods mineral composition is presented in Table 14. There was no loss of zinc in stiff porridge, unfermented kita, and besso. The loss was in anebabero (4%), biscuit (8.8%) and nifro (8.8%). Fe loss in stiff porridge, unleavened flat bread, anebabero, besso was 3.4%; in nifro (10.3%) and biscuit (6.9%). Ca in processed foods was reduced in stiff porridge (3.8%), unleavened flat bread (5.8%), nifro (12.0%), anebabero (8.2%), biscuit (9.2%) and besso (6.2%).

Table 14: Zn, Fe and Ca content of prepared foods and a raw maize (Melkasa 1)

Sample Type	Zinc mg/100g (DW)	Iron mg/100g (DW)	Calcium mg/100g (DW)
Melkasa 1	ǂ 2.5±0.0[a]	2.9±0.0[a]	29.3±0.0[a]
Stiff porridge	2.5±0.0[a]	2.8 ±0.0[b]	28.5±0.1[b]
unleavened flat bread	2.5±0.1[a]	2.8±0.1[b]	27.6±0.0[c]
Nifro	2.3±0.0[c]	2.6±0.0[d]	25.8±0.2[e]
Anebabero	2.4±0.0[b]	2.8±0.1[b]	26.9±0.1[d]
Biscut	2.3±0.0[c]	2.7±0.1[c]	26.6±0.2[d]
Beso	2.5±0.1[a]	2.8±0.0[b]	27.3±0.0[c]

Values within the same column followed by different superscripts are significantly different (P<0.05).

ǂ such values are the mean ± SD.

5.6 Reduction of Phytate in Prepared Foods form Melkasa 1

Phytate content of processed foods is presented in Table 15 that decreased significantly (P<0.05) in the foods produced from a maize variety (Melkasa 1). There was significant difference (P<0.05) among variously processed foods in reduction of phytate. When phytate content of raw maize compared with the processed foods (stiff porridge,

unleavened flat bread, nifro, anebabero, biscuit and besso) were decreased by 32.0%, 28.3%, 48.4%, 38.1%, 32.9% and 17.8% respectively.

Norhaizan & Nor (2009) reported cooking methods can reduced phytate and minerals content in foods. Phytic acid to zinc molar ratio ranged 2.7 for nifro to 3.9 for besso and phytic acid to iron molar ratio ranged 2.0 for nifro to 3.0 for besso. There was significant decrease ($P<0.05$) in phytic acid to zinc mole ratio and phytic acid to iron mole ratio during traditional foods preparation. There was significant difference ($P<0.05$) among prepared foods in both mole ratios. All foods phytate to zinc molar ratio had <15 which indicates good bioavailability of zinc. Phytate to iron molar ratio of all food was >1 that showed the bioavailability of iron is more affected by phytate content of the foods.

Table 15: Phytate and bioavailability estimates in foods and raw maize (Melkasa 1)

Sample Type	**Phaytate content mg/100g**	**Phy/Zn molar ratio**	**Phy/Fe molar ratio**
Melkasa 1	ǂ 121.2±0.2^{a}	4.7±0.0^{a}	3.6±0.0^{a}
Stiff porridge	82.4±0.4^{d}	3.2±0.0^{e}	2.5±0.0^{d}
unleavened flat bread	86.9±0.1^{c}	3.4±0.1^{d}	2.6±0.0^{c}
Nifro	62.6±0.5^{h}	2.7±0.0^{f}	2.0±0.0^{f}
Anebabero	75.1±0.4^{f}	3.1±0.0^{e}	2.3±0.0^{e}
Biscut	81.4±0.4^{e}	3.5±0.0^{c}	2.6±0.0^{c}
Beso	99.7±0.2^{b}	3.9±0.1^{b}	3.0±0.0^{b}

Values within the same column followed by different superscripts are significantly different ($P<0.05$).

ǂ such values are the mean ± SD.

5.7 Sensory Evaluation

The results of seven hedonic scale analysis of organoleptic characteristics of stiff porridge and unleavened flat bread is depicted in Table 16. There was no significant difference between stiff porridge and unleavened flat bread except aroma/smell.

Table 16: Mean score of sensory evaluation of stiff porridge and unleavened flat bread

Type of foods	Appearance/ Color	Test/ Flavor	Texture/ Consistency	Aroma/ Smell	Over all Acceptability
Stiff porridge	ǂ 5.6±1.1[a]	5.2 ± 1.3[b]	5.6 ± 1.0[c]	4.8 ± 1.2[d]	5.3 ± 1.1[f]
Unleavened flat bread	5.9+0.8[a]	5.5 ± 1.1[b]	5.7 ± 0.7[c]	5.6 ± 0.2[e]	5.6 ± 0.8[f]

For each column mean value with the same superscript are not significantly different (P>0.05).

ǂ such values are the mean ± SD.

6 DISCUSSION

Crops that have pro-vitamin A proved to be an effective means to alleviate vitamin A deficiency (Tumuhimbise et al., 2013). The present study revealed difference among the maize varieties in total carotenoid and β-carotene level. In white maize variety (BH 660) that was below detection limit. Highest total carotenoid (28.7± 0.3 µg/g) and beta carotene (3.1±0.1 µg/g) content were found in the melkasa 1 yellow maize variety and the lowest in Gibie Awash 1.2±0.1µg/g regarding to beta carotene and 11.4± 0.2µg/g for Melkasa7 in total carotenoid. The varied results for both parameters of yellow maize varieties were in between the tested maize varieties obtained by Safawo et al (2010) ranged 0.122 to 4.74 µg/g for β-carotene and 5.58 to 63.9 µg/g for total carotenoid. But β-carotene and total carotenoid result of this study were not in agreement with the result that was reported by Drinic et al (2014) in maize genotypes ranged 8.69 to 21.90 mg/kg for β-carotene and Dixon et al (2000) in maize hybrid total carotenoid ranged 143 to 278 µg/g. Yellow maize has the highest phenotypic variability for β-carotene, β-cryptoxanthin and α-carotene, which are carotenoids showing pro-vitamin A activity (Rios et al., 2014). Even though yellow maize contains pro-vitamin A other than β-carotene such as α-carotene and β-cryptoxantin, this study did not include their level analysis in maize varieties. As a food base strategy to fight VAD in developing countries, maize is considered as a major target crop to provide pro-vitamin A (Thompson and Amorson, 2014). Maize is staple food in Ethiopia (Mosisa et al., 2011) that helps to use it as food base approach to alleviate VAD.

Melkasa 1 maize variety had superior in β-carotene (3.1±0.1µg/g) was used to study β-carotene retention in traditionally prepared foods. β-carotene retention of this study compare to the results which was reported by Li et al (2007) on β-carotene retention of fermented porridges (75.5%) and unfermented porridges (75.2%) β-carotene retention, in this study in stiff porridge (88.9%), unleavened flat bread (88.3%), anebabero (80.5%) were higher. All traditional foods in the present study β-carotene retention had higher than the investigation of Ejigui et al (2005) on retention of β-carotene after four days fermentation of yellow maize (39%). In addition, processing of six types of traditional

foods affects other nutritional composition. The difference in β-carotene retention and other nutrient change among traditional foods could be the difference in cooking methods, time, temperature and varied sequences.

During roasting and backing, nutrients undergo chemical changes resulting in a reduction of their nutritional value (Sarwar et al., 2012). Carotenoids in dehydrated products are more likely to undergo degradation (Fennema, 1996) that may be one of the causes for besso lower beta carotene retention (55.6%). During all foods preparation except besso the raw was constitutes with water before exposing to heat. In addition, roasting can reduce the moisture content of raw maize that may be enhancing the degradation of β-carotene during milling.

β-carotene retention of unleavened flat bread (88.3 %) was higher than anebabero (80.5%). Both foods were cooked by baking using traditional mitad but anebabero was fermented before baking. During food fermentation, raw materials undergo overall changes in composition, flavor and textural properties. It causes complex changes to proteins and carbohydrates that soften the texture of fermented products. Fermentation also contributes a decrease in pH preserve foods by production of acids (Fellows, 2000). The softening of the texture of the dough during fermentation may be the cause of β-carotene to be easily exposed to baking temperature and that may enhance degradation.

Percentage provide for daily estimated mean requirement intake (µg RE/day) of vitamin A for different age and gender group per consumption of 100g of each food contributed by stiff porridge (23.3 to 77%), unleavened flat bread (22.5 to 75%), anebabero (20.8 to 69.4%), nefro (20 to 66.7%), biscut (17.5 to 58.3%) and besso (14.2 to 47.2%).

Yellow maize varieties in the present study had greater or at least comparable amount of crude fat (6.1 vs 5.3%), crude protein (12.3% vs 12.1%), total ash (1.6% vs 1.4%), fiber (2.3% vs 1.8%), carbohydrate (67.5% vs 67.7%), Fe (3.1 mg vs 2.4mg), Zn (2.6mg vs 2.5mg), Ca (33.7 mg vs 31.3 mg) to white maize variety. Yellow maize is basically comparable to white maize except for its grain color due to the presence of carotenoids (Egesel et al., 2003). The variability in carbohydrates, protein, fats, ash, crude fiber,

moisture and mineral content of maize varieties may be due to genetics and environmental factors (Ullah et al., 2010).

Phaytate can adversely affect the bioavailability of kernel minerals essential for human health such as calcium, magnesium, iron and zinc and can therefore contribute to mineral deficiencies (Coulibaly et al., 2011). In the present study, the white maize variety had 3.7 phytate to zinc molar ratio and 3.4 phytate to iron molar ratio. Phytate to zinc molar ratio was 3.8 and phytate to iron molar ratio was 2.9 for yellow maize varieties. Phytate to zinc molar ratio of yellow maize and white maize was <15 which indicates good bioavailability of zinc. Phytate to iron molar ratio of both varieties was >1 that showed the bioavailability of iron is affected by phytate content. This suggests that in addition, their superior to β-carotene, yellow maize varieties have comparable Zn and Fe bioavailability. Bioavailability of Fe was also affected by phytate in all traditional foods.

De Groote and Kimenju (2008) reported in Kenya poor acceptance of yellow maize comes from prejudice and negative associations, such as food aid and animal feed, rather than from sensory characteristics such as taste. Even though, yellow maize has high provitamin A and non provitamin A carotenoid than white maize, in Ethiopia consumption of yellow maize for food is lower compared to white maize. Therefore, there is a need to promote yellow maize by developing sensory acceptable products. In the present study, sensory acceptance was conducted for stiff porridge and unleavened flat bread. The result showed that both foods sensory parameters values were equal to or greater than value of like moderately.

7 CONCLUSION AND RECOMMENDATION

7.1 Conclusion

Staple foods in Ethiopia such as yellow maize can be more accessible than other like fruit and vegetable for lower income society. Yellow maize contains good amount of β-carotene that can be one of the target crops to implement inexpensive and sustainable food base strategy to alleviate VAD. The present study showed that yellow maize is superior in β-carotene content and had comparable amount of other nutrients and bioavailability of Zn and Fe to white maize. In addition, it provides antioxidants that lower risk of cardiovascular disease, cancer, cataracts and age related macular degeneration.

In order to combat vitamin A deficiency by enhancing consumption of yellow maize, evaluation on the maize food preparation in the retention of β-carotene is an important step to recommending and promoting the food to consumer. This study revealed difference among cooking methods regarding retention of β-carotene, proximate and mineral composition and reduction of phytate. The highest retention of β-carotene was seen in stiff porridge (88.9%) and unleavened flat bread (88.3%). The foods were prepared from maize through the number of steps and the varied sequences that can be responsible for observed difference in retention.

Stiff porridge and unleavened flat bread were selected for sensory analysis. Both foods were acceptable in sensory wise that foods may be superior foods to provide better beta carotene to the society from yellow maize. This could be information for consumers to decide on a cooking practice and a type of maize to use and improve the nutritional quality of their foods. From this study it is possible to see that if the yellow maize is prepared in the form of Stiff porridge and unleavened flat bread it is possible to get reasonable amount of β-carotene.

7.2 Recommendation

All stakeholders who involve in combating VAD in the country should involve and take part to promote and provide nutrition education on the benefits of yellow maize.

Further work is needed that improve color of food prepared form yellow maize variety without compromising the β-carotene content.

Optimization of cooking temperature and time to find optimum cooking condition may need further study.

There is an opportunity to increase β-carotene and other provitamin A in maize through biofortification. Agricultural sector should play role to enhance the production and accessibility of yellow maize with high β-carotene content.

Foods with β-carotene should be accompanied with fat to enhance its bioavailability.

8 REFERENCES

Abdo E.M., Barbary O.M. and Shaltout O.E (2013). Chemical analysis of BT corn "Mon-810: Ajeeb-YG" and its counterpart non-Bt corn "Ajeeb". *IOSR Journal of Applied Chemistry*, 4; 55-60.

Abebe, M., Liu W., White W.S., Dixon B.M., Rocheford T. (2008). Carotenoid diversity in tropical-adapted yellow maize inbred lines, *Food Chemistry* 109; 521–529.

Adem O. S., Singh P. and Berhanu G. (2012) Assessment of Dietary Consumption of Vitamin A by Preschool Children in Southern Ethiopia- A Cross Sectional Study. *International Journal of Environmental Sciences* 1; 279-284.

Adom, K. K. and Liu R. H.(2002). Antioxidant activity of grains. *Journal of Agriculture Food Chemistry*, 50; 6182–6187.

African Union (2005). African Regional Nutritional Strategy (2005–2015). http://www.African-union.org.

Allen L., Benoist B.D., Dary O., Hurrell R.(2006). Guidelines on food fortification with micronutrients, WHO and FAO

Aluru M., Xu Y., Guo R., Wang Z., Li S., White W., Wang K., Rodermel S. (2008). Generation of transgenic maize with enhanced provitamin A content, *Journal of Experimental Botany*, 59; 3551–3562.

Aman R., Schieber A. and Carle R. (2005). Effects of heating and illumination on trans-cis isomerization and degradation of carotene and lutein in isolate spinach chloroplasts. *Journal of Agriculture and Food Chemistry,* 53; 9512-9518.

AOAC (2000), American Official Chemist Method, Washington , USA.

Asrat W. (2011) Development of Suitable Processes for Some Ethiopian Traditional Foods Using Quality Protein Maize: Emphasis on Enhancement of the Physico-Chemical Properties. Ethiopian Health and Nutrition Research Institute (EHNRI), Proceedings of the Third National Maize Workshop of Ethiopia, 18-20 April 2011 Addis Ababa, Ethiopia (pp.260-267). EARO (Ethiopian Agricultural Research Organization) and CIMMYT (International maize and wheat Improvement Center).

Belfield S. and Brown C. (2008) *Field crop manual: maize a guide to upland production in Cambodia*. 1st Edition.

Berdanier C. D. (1998) *Advanced Nutrition Micronutrients*. Boca Raton, London, New York Washington, D.C.

Berhanu G., Rivera F.S., Mohammed H., Mwangi W. and Seid A. (2011) *Maize and livestock: Their inter-linked roles in meeting human needs in Ethiopia*. Research Report 6, ILRI (International Livestock Research Institute) Addis Ababa, Ethiopia.

Bhupender K. et al (2012) *Maize biology: An introduction*. Indian Council of Agricultural Research (ICAR), Technical bullettin.

Bibiana I., Grace N. and Julius A. (2014) Quality Evaluation of Composite Bread Produced from Wheat, Maize and Orange Fleshed Sweet Potato Flours. *American Journal of Food Science and Technology*, 2; 109-115.

Bouis H.E and Welch R.M.(2010) Biofortification-A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science,* 50; 20-32.

Coulibaly A., Brou K. and Jie C.(2011). "Phytic acid in cereal grains: Structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional qunality, *American Journal of Plant Nutrition and Fertilization Technology*, 1; 1-22.

De Groote H. and Kimenju S.C. (2008). Comparing consumer preferences for color and nutritional quality in maize: application of a semi-double-bound logistic model on urban consumers in Kenya. *Food Policy*,33;362-370.

De Oliveira, P. R. G.and Rodriquez-Amaya, B. D. (2007) Processed and prepared corn products as sources of lutein and zeaxanthin: compositional variation in the food chain, *Journal of Food Science* 72; 79-85.

Demissie T, Ali A, Mekonen Y., Haider J. and Umeta M. (2010) Magnitude and distribution of vitamin A deficiency in Ethiopia. *Food and Nutrition bullet,* 31; 334-241.

Demissie T, Ali A, Mekonen Y., Haider J. and Umeta M. (2009) Demographic and health related risk Factors of subclinical VAD in Ethiopia. *Journal Health, Population and nutrition, 27; 666-673.*

Dewanto. V., Wu, X. Z., Liu, R. H. (2002) Processed sweet corn has higher antioxidant activity. *Journal of Agriculture Food Chemistry*, 50; 4959– 4964.

Dixon B. M. (2000) Genetic variation in total carotene, iron, and zinc contents of maize and cassava genotypes, *Food and Nutrition Bulletin*, 21; 419-422.

Drinic S.M., Vesna D., Sladana Z., Zorica B. and Dragan K. (2014). Variability of tocopherol and beta carotene. *Journal of International Scientific Publications: Agriculture and Food,* 2; 192-198.

Egesel, C.O., J.C. Wong R.J. Lambert, and T. Rocheford. (2003). Combining ability of maize inbreds for carotenoids and tocopherols. *Crop Science*, 43; 818–823.

Ejigui J., Savoie L., Marin J. and Desrosiers T. (2005). Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antnutritional factors of yellow maize (Zea mays). *Journal of Biological Sciences* 5; 590-596.

Enyisi .I. S. (2014). Chemical and nutritional value of maize and maize products obtained from selected markets in Kaduna State, Nigeria. *African Journal of Food Science and Technology* ((ISSN: 2141-5455), 5; 100-104.

Ethiopia Commodity Exchange Authority (ECXA) (2009). *Understanding maize: A review of supply and marketing issues.* Addis Ababa, Ethiopia.

Ethiopian standard: ES 679:2001 *Maize (Corn) specification.* 1st Edition, Ethiopian standards Agency, Addis Ababa, Ethiopia.

Ethiopian standard: ES ISO 5498:2002, *Agricultural food products- Determination of crud fiber content- General method.* 1st Edition, Ethiopian Standards Agency, Addis Ababa, Ethiopia.

(FAO) Food and Agricultural Organization of the United Nations (2012), http://faostat3.fao.org.

(FDA) Food and Drug Administration (1994), *Validation of chromatographic method*.

Fardet A., Rock E. and Remesy C. (2008). Is the in vitro antioxidant potential of whole-grain cereals and cereal products well reflected in vivo? *Journal of Cereal Science*, 1–19.

Fellows P.J. (2000). *Food processing technology: principle and practice*. 2nd Edition, Washington DC, Woodhead publishing limited.

Fennema O. R. 1996). *Food chemistry*. 3rd Edition, New York: Marcel Dekker Inc.

Fofanh M. Fofanah, BA,BSN,RN and MPH (2011). Communication strategy for the promotion of orange fleshed sweet potatoes in Tigray, Ethiopia. *International Potato Center*,1-24.

Gibney M.J et al (2009). *Introduction to human nutrition*. 2nd Edition, United Kingdom,The Nutrition Society, A John Wiley & Sons, Ltd.

Gwirtz J.A and Garcia-Casal M.N. (2014). Processing maize flour and corn meal food products. *Annals of the New York Academy of Sciences*,1312; 65-75.

Harvey R. A. and Ferrier D. R. (2011). *Lippincott's illustrated reviews: Biochemistry*. 5th Edition, China, Lippincott Williams & Wilkins.

Hedren E., Diaz V. and Syanberg U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *European Journal of Nutrition*,56; 425-430.

Hoopen T. and Maiga A. (2012). *Maize production and processing*. Cameroon.

Howe J.A. and Tanumihardjo S.A. (2006). Carotenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. *Journal of Nutrition*, 136; 2562– 2567.

Ijabadeniyi, A.O. and Adebolu, T.T. (2005). The effect of processing methods on the nutritional properties of ogi produced from three maize varieties. *Journal of Food Agriculture and Environment*, 3; 108-109.

James C. (2003). Global review of commercialized transgenic crops: 2002 feature: Bt maize . ISAAA Briefs, 29.

Jim M. and Truswell A. S. (2002). *Essentials of human nutrition*. 2nd Edition, New York, Oxford University.

Kavitha S. and Parimalavlli R. (2014). Effect of processing methods on proximate composition of cereal and legume flours, *Journal of Human Nutrition and Food Science*, 1051; 1-5.

Kean E.G, Hamaker B.R, Ferruzze M.G. (2008). Carotenoid bioaccessibility from whole grain and degermed maize meal products. *Journal of Agriculture and Food Chemistry*, 56; 9918–9926.

Kumar U. and Kweera B. (2014). Comparative analysis of nutritional value and aflatoxin level of maize grain from different site of Rajasthan, *International Journal of Scientific and Technology Research*, 2; 333-335.

Li S.,Tayie F.A.K.,Young M.F.,Torbert R. and White W.S. (2007). Retention of provitamin A carotenoids in high carotene maize (Zea mays) during traditional African household processing. *Journal of Agriculture and Food Chemistry*,55;10744-10750.

Li S., Nugroho A., Rocheford T. and White W.S. (2010). Vitamin A equivalence of the β-carotene in β-carotene–biofortified maize porridge consumed by women, *The American Journal of Clinical Nutrition* 92; 1105–1112.

McGiore M. and Beerman K.A (2011). *Nutritional sciences from fundamentals to food,* 2nd edition, USA, Wadsworth Cengage Learning.

McKevith B. (2004). Nutritional aspects of cereals. *British Nutrition Foundation Nutrition Bulletin*, 29; 111–142.

M'mboyi F. Mugo S., Mwimali M., Leah Ambani L. (2010). *Maize production and improvement in sub-saharan Africa*. Nairobi:African Biotechnology Stakeholders forum (ABSF).

Mosisa W., Legesse W., BirhanuT., Girma D., Girum A., Wende A., Tolera K., Gezahegn B. (2011). Status and future direction of maize research and production in Ethiopia. Proceedings of the Third National Maize Workshop of Ethiopia, 18-20 April 2011 Addis Ababa, Ethiopia (pp.17-23). Addis Ababa: EARO (Ethiopian Agricultural ResearchOrganization) and CIMMYT (International and wheat Improvement Center).

Mozaffarieh M., Sacu S. and Wedrich A. (2003). The role of the carotenoids, lutein and zeaxanthin, in protecting against age related macular degeneration: a review based on controversial evidence. *Nutritional Journal*, 2; 1-8.

Muzhingi T. (2008). Determination of carotinoid in yellow maize, the effect of saponification and food preparation. *Journal of the Federation of American societies for experimental Biology*, 78; 112-120.

Muzhingi T., Langyintuo A.S., Malaba L.C., Banziger M. (2008). Consumer acceptability Consumer acceptability of yellow maize products in Zimbabwe of yellow maize products in Zimbabwe. *Food Policy*, 33; 352–361.

Muzhingi T., Gadaga T.H., Siwela A.H., Grusak M.A., Russell R.M. and Tang G. (2011). Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men, *American Journal of Clinical Nutrition*, 94; 510–519.

Ndukwe O.K., Edeoga H.O., Omosun G. (2015). Varietal differences in some nutritional composition of ten maize (Zea mays L.) varieties grown in Nigeria. *Nutritional Journal of Academic Research and Reflection*, 3; 1-11

Norhizan M.E. and Nor F.A (2009). Determination of phytate, Iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malaysia Journal of Nutrition*, 15; 213-222.

Nuss E.T., Tanumihardjo S.A (2010). Maize: a paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety* 9; 417-436.

Oladeji A.E., Maziya-Dixon B., Abebe M., Olaofe O. and Irondi E.A. (2015). Effect of maturity stages and roasting method on the proximate composition of orange maize hybrids. *Global Advance Research Journal of Agricultural Science*, 4; 462-468.

Queiroz V.P., Guimaraes P.E., Queiroz L.R., Guedes E.D.,Vasconclos V.D., Guimaraes L.J., Ribeiro P.E. and Schaffer R.E. (2011). Iron and zinc availability in maize line, 31; 577-583.

Rios A.R., Paes M.C., Cardoso W.S., Borem A. and Teixeira F. (2014). Color of corn grains and carotenoid profile of importance for human health. *American Journal of Plant Sciences*, 5; 857-862.

Rodriguez-Amaya D.R. and Kimura M. (2004). *Harvestplus handbook for carotenoid analysis*. HarvestPlus Technical Monograph, Washington, DC.

Rodríguez-Amaya, D. B. (1997) *Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed, and stored foods. Opportunities for Micronutrients Interventions (OMNI) Project*. Arlington, Va., U.S.A.: John Snow, Inc.

Ruel M. T. (2001). Can food-based strategies help reduce vitamin A and Iron deficiencies? A Review of Recent Evidence. International Food Policy Research Institute Washington, D.C.

Safawo T., Senthi N., Raveendran M., Vellaikumar S. Ganesan K.N., Nallarhambi G., Saranya S., Shobrana V.G., Abirami B. and Gowri E.V. (2010). Exploitation of natural variability in maize for β - Carotene content using HPLC and gene specific markers. *Journal of Plant Breeding*, 1; 548-555.

Sanders T. and Emery P. (2003). *Moleculer basis of human nutrition*. London, King's College,Department of Nutrition and Dietetics.

Saltzman A., Birol E., Bouis H.E., Boy E., Demoura F.F., Islam Y. and Pfeiffer W.H. (2014). Biofortification: Progress toward a more nourishing future. *Pontifical Academy of Sciences*, 125; 1-23.

Sanusi R.A. and Adebiyi A. (2009). Beta carotene content of commonly consumed foods and soups in Nigeria. *Pakistan Journal of Nutrition*, 8; 1512-1516.

Sarwar G.G., Wu X.C. and Kockell K. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. British Journal of Nutrition, 108; 315-332.

Schlemmer U., Frolich W., Prieto R.M. and Grases F. (2009). Phytate in food and significance for humans: food sources, intake, processing, bioavilability, protective role and analysis, *Molecular Nutrition and Food Research*, 53; S330-S375.

Schneider K. and Anderson L. (2010) Yield gap and productivity potential in Ethiopian agriculture: Staple grains & pulses. *Evans School Policy Analysis and Research (EPAR)*, 98; 6-24.

Serrem C. A., de Kock H.L. and Taylor J. R. N. (2011). Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *International Journal of Food Science and Technology*, 1- 26.

Sesso, H. D.et al (2004). Plasma lycopene, other carotenoids and retinol and the risk of cardiovascular disease in women. *American Journal of Clinical Nutrition*, 79; 47−53.

Sommer A.(1995). *Vitamin A deficiency and its consequences*: *A field guide line to detection and control*, 3rd edition, Geneva, WHO.

Thompson B. and Amoroso L. (2011). *Combating micronutrient deficiencies: Food-based approaches.* Italy, FAO.

Thompson B. and Amoroso L. (2014). *Improving diets and nutrition: Food based approaches*. Rome, FAO and Wallingford, UK, CABI.

Tumuhimbise G. A., Nanutebi A., Florence T. and John M. (2013). Provitamin A crops: acceptability, bioavailability, efficacy and effectiveness. *Food and Nutrition Sciences*, 4; 430-435.

United Nations System Standing Committee on Nutrition (SCN) (2005). 5th Report on the world nutrition situation: Nutrition for Improved Development Outcomes.

Ullah I., Ali M. and Farooqi A. (2010). Chemical and nutritional properties of some maize (Zea mays L.) varieties grown in NWFP, Pakistan. *Pakistan Journal of Nutrition* 9; 1113-1117.

USDA-Nutrient database (2013).www.http://ndb.nal.usda.gov/ndb/search/list.

Whitney E and Rady S. (2008) *Understanding Nutrition*, 11th Edition, USA: Thomson Learning Inc.

WHO (2003).GEMS/Food regional diets: regional per capital consumption of raw and semi-processed agricultural commodities, food safety department. WHO document production services, Geneva, Switzerland.

WHO (2009). *Global prevalence of vitamin A deficiency in populations at risk 1995–2005 WHO global database on vitamin A deficiency*. Geneva, Switzerland: WHO.

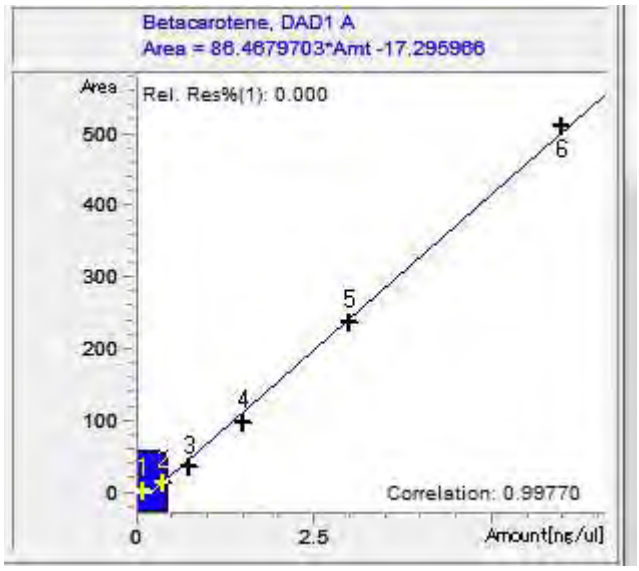
WHO (2011). *Vitamin A supplementation in infants 1–5 months of age*. Geneva, Switzerland: WHO.

WHO/FAO (2004). *Vitamin and mineral requirements in human nutrition*. 2nd Edition, Switzerland: WHO.

WHO/FAO (2006). *Guidelines on food fortification with micronutrients*. Geneva, Switzerland: WHO.

WHO/WFP/UNICF (2006). Preventing and controlling micronutrient deficiencies in population affected by an emergency.

Yeung D. L. and Laquatra I. (2003). *Heinz Handbook of Nutrition*. 9th Edition, H.J., Heinz Company.


Betacarotene, DAD1 A
Area = 86.4679703*Amt -17.295966
Area
Rel. Res%(1): 0.000
500
400
300
200
100
0
1
2
3
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6
Correlation: 0.99770
0
2.5
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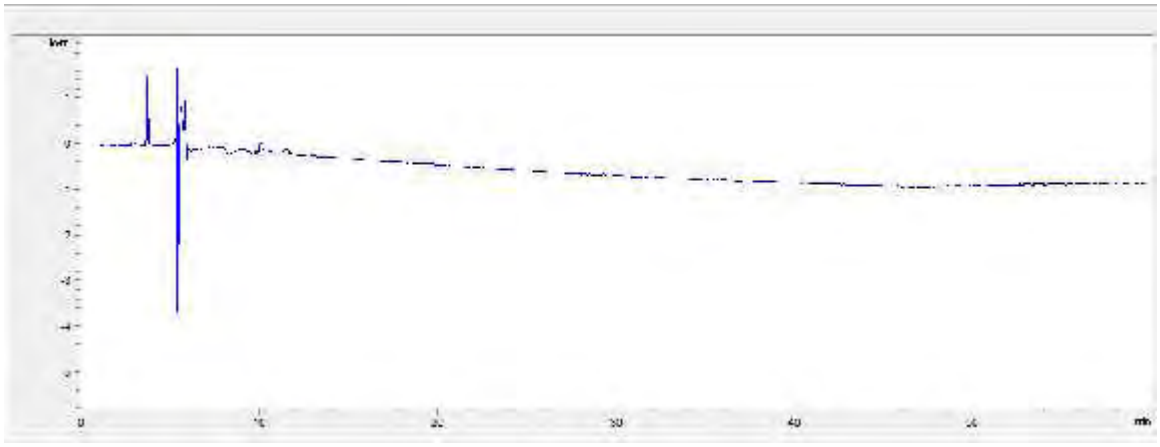

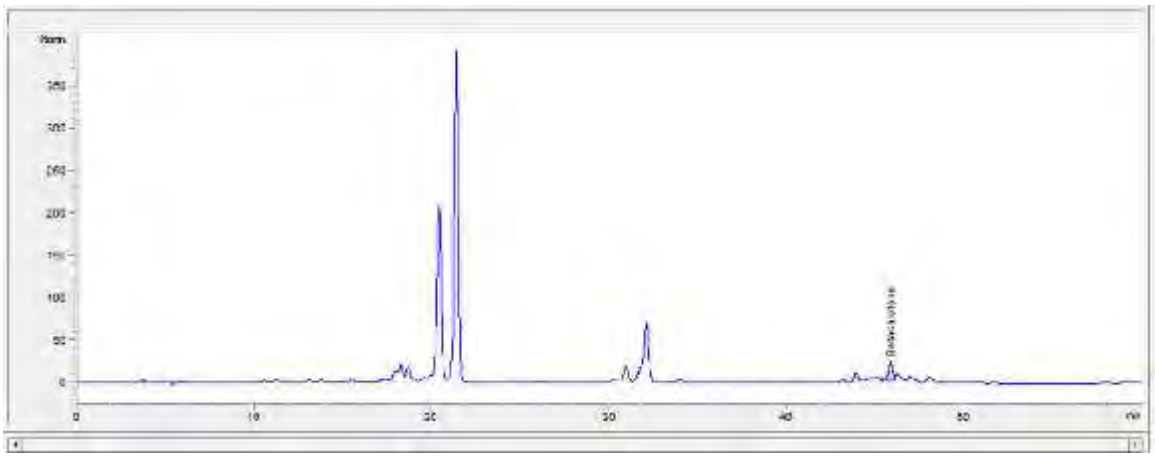

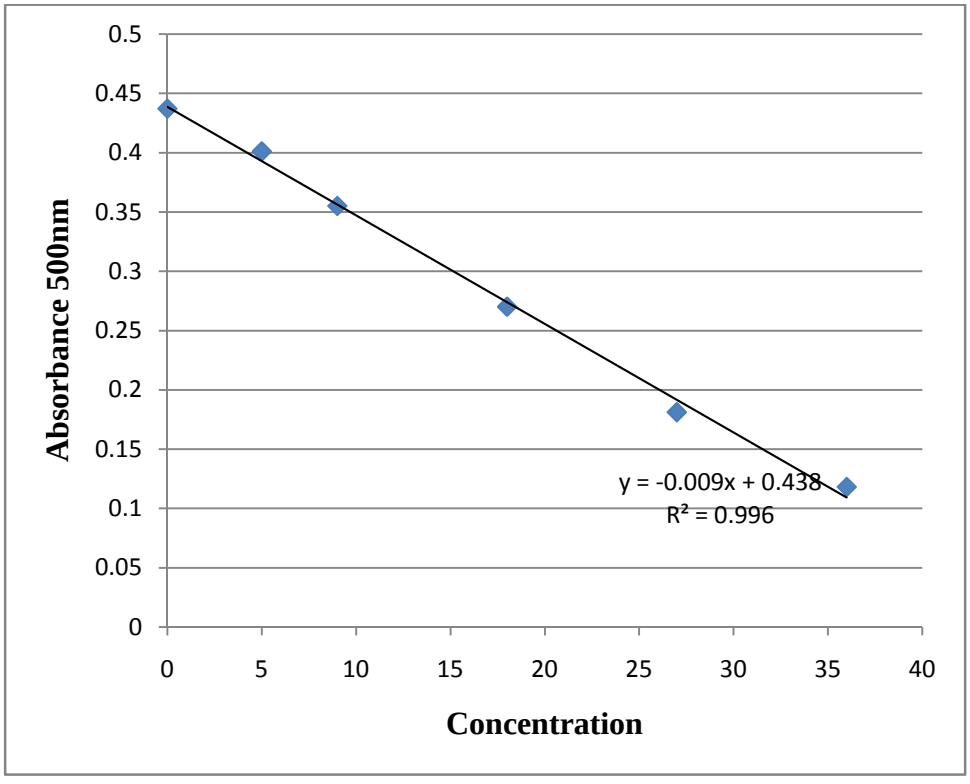
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Absorbance 500nm
0
5
10
15
20
25
30
35
40
Concentration
y = -0.009x + 0.438
R² = 0.996

Appendix 5: Score sheet for sensory evaluation

Panelist Code…… Sample Code……. Date……..

Directions: Check one rating for each of the following: Appearance, Taste/Flavor, Texture/Consistency, Aroma/Smell and Overall Acceptability					
Rating Scale	Appearance/Color	Taste/ Flavor	Texture/ Consistency	Aroma/ Smell	Overall Acceptability
7. Like Extremely					
6. Like Very Much					
5. Like Moderately					
4. Neither Like or Dislike					
3. Dislike Moderately					
2. Dislike Very Much					
1. Dislike Extremely					
Thank You					