Effect of processing on bioaccessibility and antioxidant capacity of carotenoids from maize

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ABSTRACT

This study has assessed the effect of processing (by boiling, roasting, and fermenting and through the production of fermented porridge and unfermented porridge) on bioaccessibility and antioxidant capacity (oxygen radical absorbance capacity (ORAC) and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity) of carotenoids (lutein, zeaxanthin, β–cryptoxanthin, α-carotene, and β-carotene) from maize. High performance liquid chromatography was used to analyze the content of carotenoid compounds, and digestion of maize samples was done using a three-stage stimulated human digestion system. On average, boiled maize had the highest retention rate of 79.0%, followed by fermented porridge (73.8%), fermented maize (68.4%), and unfermented porridge (59.0%), while roasted maize had the lowest retention rate of 56.1%. The bioaccessibility of carotenoids was analyzed using a three-stage in-vitro digestion. Without any cooking treatments, carotenoids recovery from raw maize ranged from 73.0-100.5%. After cooking treatments, carotenoids recovery showed varying levels of increase except for fermented maize. The average recovery of carotenoids in whole digesta following boiled maize, roasted maize, fermented porridge and unfermented porridge were 108.5%, 116.9%, 101.9%, and 110.7%, respectively.

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1. INTRODUCTION

Maize is one of the most important crops in the world, mainly utilized as food, feed, and industrial raw material (Thakkar, Maziya-Dixon, Dixon, & Failla, 2007; Watson, 2003). Among the cereals worldwide, maize takes third place among the staple foods after wheat and rice, except in African countries where maize is served as the main staple food (Watson, 2003). Maize is usually classified based on its color (yellow/ white) and caryopsis shape (dent/flint). The most common type is yellow-dent. Maize can be planted under various soil conditions and environments (Serna-Saldivar, 2016).

Maize provides energy, serving as a rich source of micronutrients such as phenolic acids and carotenoids (Kean et al., 2008). Vitamin A deficiency has become a public health problem, especially in some developing countries, including those in Africa and Southeast Asia. Consumption of food devoid of carotenoids could be related to this health issue. Carotenoids could play an important role as bioactive compounds. Lutein and zeaxanthin make up almost 70% of the total found in maize. Both α- and β-carotene, as well as β-cryptoxanthin, are considerably less present in the maize kernel.

Maize has various kinds of colors ranging from white, yellow, orange, and purple, some of which are illustrative of the type of constitutive bioactive compounds. For example, yellow maize has a higher level of carotenoids and antioxidant activity than white maize (de la Parra et al. 2007).

Micellarization efficiency of carotenoids is defined as the percentage of carotenoids subsequently transferred from the crude digesta to the filtered aqueous fraction during digestion. Micellarization efficiency is commonly used as a measure of relative bioaccessibility which correlates to carotenoid bioavailability in-vivo (Failla, Chitchumroonchokchai, & Ishida, 2008). Bioavailability is defined as the fraction of carotenoids which can be absorbed from a food source by tissue and circulated in our system. During this process, bioaccessibility is defined as proportion of carotenoids in mixed micelles from a food source (Kean et al., 2008).

In this study, two experimental chapters were included. The objective of the first experimental chapter was to examine the effects of different maize-based products, including boiled maize, roasted maize, fermented maize, fermented porridge, and unfermented porridge, on carotenoid contents and composition. The second experimental chapter focused on the bioaccessibility of carotenoids of these five maize-based food products and their antioxidant capacity.

2. LITERATURE REVIEW

2.1 Cereal and maize production

Since 2014, total production of cereals in the world has exceeded 2.7 billion tons (FAOSTAT 2017). Rice (*Oryza sativa*), wheat (*Triticum aestivum*), and maize (*Zea mays ssp. mays*) are the three main grains among cereals, yielding almost 87% of total cereal production (Matsuoka, Yamazaki, Ogihara, & Tsunewaki, 2002). The global maize production during 2014 was 1,038 million tonnes (mt) within an area of 185 million hectares (mha), making it the second largest produced among agricultural commodities and followed by sugar cane throughout the world (FAOSTAT 2017). The USA, China, Brazil, Mexico, Argentina, India, France, Indonesia, South Africa, and Ukraine are the top ten maize-producing countries in the world. Compared with the other two main crops in 2014, throughout of world, rice production measured 741 million tonnes (mt) within an area of 163 million hectares (mha) and wheat world production measured 729 million tonnes (mt) within an area of 220 million hectares (mha) (FAOSTAT 2017).

2.2 Structure and general characteristics of maize

The endosperm occupies the largest part of the maize kernel, making up 80 to 85 percent. The endosperm consists of numerous cells which are packed with a mixture of starch granules and protein matrix. In addition, the cell wall is composed of proteins, non-starchy polysaccharides, phenolic acids, and carotenoids (Watson, 2003). Maize is considered a high carotenoid cereal with the carotenoids in corn endosperm ranging from 2 to 32 mg/kg. Compared with other cereals, wheat ranges from 1.5 to 10 mg/kg and oats from 0.5 to 1.2 mg/kg in carotenoid levels (Ndolo & Beta, 2014; Watson, 2003). Starchy endosperm, consisting of nutrients needed during germination, occupies about 70 percent of the kernel's weight.

The germ is the second largest part of the maize kernel which composes about 9 to 10 percent of its weight. Instead of a large amount of starch, the germ is composed of a high level of fat (about 33%), relatively high protein (18-19%), and some minerals (Watson, 2003). The fatty acids in germ are mainly oleic and linoleic, and these unsaturated fatty acids improve the antioxidant capacity and therefore make the oil in germ more stable.

The bran can be separated into two major layers: pericarp as the outer layer of the maize kernel which contains high levels of crude fiber, such as cellulose, lignin and hemicellulose, and inner aleurone layer (Moreau, Singh, Nunez, & Hicks, 2000; Watson, 2003). The aleurone layer is also the outer part attached to the starchy endosperm and usually removed as part of bran during milling. The aleurone in maize consists of a single layer of thick-walled cell with dense contents and prominent nuclei (Moreau et al., 2000). The aleurone layer contains high levels of bioactive compounds, including soluble and insoluble dietary fiber, such as xylans, β-glucans, raffinose, stachyose, and fructans. It also contains antioxidants, such as phenolic acids, carotenoids, lignans, anthocyanins and isoflavonoids, vitamin E, vitamin B groups, minerals, phytic acid, enzymes, betaine, and choline (Amrein, Gränicher, Arrigoni, & Amadò, 2003; Masisi et al., 2015; Ndolo & Beta, 2014).

The maize kernel, especially the germ, contains several bioactive compounds, such as anthocyanins, phenolic compounds, tocopherols, and carotenoids. Naturally, maize is rich in carotenoids, such as β-carotene, lutein, cryptoxanthin, and zeaxanthin. These bioactive components can help people maintain normal vision, lower their oxidative stress, and prevent some diseases, such as cardiovascular disease, type 2 diabetes, and cancer (Thakkar, Maziya-Dixon, Dixon, & Failla, 2007); Panfili et al. 2004; De Oliveira & Rodriquez-Amaya 2007).

2.3 Maize utilization

As one of the main cereal crops, maize has been processed and consumed in different ways. Nowadays, although household and industrial foods that incorporate maize are increasing, more than 85% of maize is used for feed and bioethanol production. Wet milling, dry milling, and nixtamalization are the most commonly used approaches for maize processing in the industry (Serna-Saldivar, 2016). For industrial use, maize can be wet milled to produce pure starch as the main product and protein, oil, fiber and germ as coproducts. These components can then be used for industrial products, such as sweeteners, corn oils, corn starch, and beverages (Watson, 2003). Corn starch can also be utilized as a raw ingredient in industry to make corn syrup and corn alcohol for food or to manufacture plastics and adhesives for non-food usage. In the past decades, due to the increased demand of sweeteners in the soft drink industry, the demand of maize syrups has increased.

Maize can also be dry milled to produce corn grits, corn meal, and subsequently snack and breakfast cereal. During milling, some mills will remove coarse parts of bran and germ while other mills will maintain the whole meal. The advantage of preserving whole meal is that it has higher nutritional value, such as higher content of fiber and lipids. On the other hand, whole meal maize products have a shorter shelf life because of the tendency for the oil therein to undergo rancidity. In developing countries, maize is usually grown for direct human consumption. People usually grind and pound soaked maize. Then maize meal can be cooked into various kinds of traditional food products, such as Chichi, tesguinio, ogi, and nsima. Chichi is a kind of alcoholic beverage which contains 2-12% alcohol. Steinkraus (1995) has reported that chichi contains several beneficial microorganisms and is rich in vitamin B. Ogi maize-based porridge is widely consumed in West Africa, and it is a typical fermented food. Ogi porridge is rich in protein, vitamins, minerals, and some microorganisms such as lactic acid bacteria, aerobic bacteria, and yeast which improve its nutritional and functional value (Achi, 2005; K. H Steinkraus, 1995).

Nixtamalization of whole maize has been used more and more widely in industry. It can be used to produce lime-cooked and alkaline maize, as well as corn and tortilla chips. Table or soft tortillas were commonly consumed in developed countries, where they became more and more popular in the United States and some other countries as part of the consumption of Mexican cuisine; soft tortillas were also a staple food for people in developing Latin American countries and Africa countries (Serna-Saldivar, 2016).

Whole maize is also widely used to produce popcorn and corn nuts. Popcorn is currently one of the most popular snacks. There are millions of processors of popcorn around the world. Popcorn is usually ‘popped’ using yellow-flint corn which contains mostly corneous endosperm and hard pericarp. These properties allow the internal temperature and pressure to increase high enough to ‘pop’ the kernels (Karababa, 2006; Regev & Nisan, 2000). Roasting is one of the traditional dry heat processing methods. Roasting usually leads to some chemical, aroma, and color changes. Due to the higher temperature used and the dry conditions, roasting could lead to a reduction of carbohydrate and proteins because of the oxidative reactions (Karababa, 2006) Some studies also reported micronutrient loss during roasting. Comparing roasted maize with raw maize, phytic acid phosphorus was reduced from 146±9.44 to 123±8.20 mg/100g DW, tannin reduced from 30.9±2.02 to 15.1±2.52 mg/100g DW, and hydrocyanic acid reduced from 2.20±0.18 to 1.44±0.16 mg/100g DW. Minerals such as potassium (13.8%) and calcium (41.1%), carotene (24.7%), riboflavin

(32.4%), ascorbic acid (35.1%), and thiamine (26.8%) also showed a reduction after roasting (Agume Ntso, Njintang, & Mbofung, 2017; Ayatse, Eka, & Ifon, 1983).

2.4 Porridge making and fermentation

Throughout African countries, porridge is a major traditional food cooked using maize. Fermented maize can be used to complement breast milk for infants in some countries. According to the National Food Consumption Survey (NFCS) in South Africa, more than 90% of children between ages 1 and 9 years old consume maize porridge.

To make porridge, people in African countries usually ferment the soaked maize. Fermentation is a traditional step of cereal cooking (Jespersen 2003). It is also one of the most economical ways to preserve food. Fermentation helps to destroy undesirable components while improving the nutritional value and flavor of food. In addition, fermentation helps to reduce energy needed for further processing and reduce the product volume for transportation (A. Blandino, Alaseeri, Pandiella, Cantero, & Webb, 2003).

Different fermentation techniques, raw materials, and microorganisms make fermented products abundant. Alcohol, lactic acid, acetic acid, and alkali are the four main fermentation byproducts (A. Blandino et al., 2003). For example, alcohol fermentation can produce ethanol using yeast as the predominant organisms, a process widely applied in the wine and beer industries. In the last decade, fermentation has gained popularity worldwide as a home art to make products like flavored fruit beverages, soy sauce, and homemade yogurt.

Fermentation is practiced in developing countries to enhance the bioavailability of micronutrients for infants and children because it can provide an optimal pH for enzyme degradation of phytate which could increase the solubility of calcium, iron, and zinc (Hotz & Gibson 2007). Although most fermentation techniques require the addition of exterior microorganisms, natural fermentation utilizes the raw materials' own microorganisms, such as *Lactobacillus fermentum* and *Lactocillus brevis* (Agume Ntso et al., 2017). Natural fermentation is usually employed to prepare porridge in the household. During fermentation, synthesis of certain amino acids occurs, while antinutrients, total polyphenolic compounds, and vitamin B complex synthesis decreases and the pH could drop from 6.1 to 3.6. A pH decrease can provide a better environment for the growth of lactic acid bacteria. A pH drop can also stimulate phytate enzymatic degradation which may increase the availability of certain mineral such as zinc, iron, and calcium (K. H. Steinkraus, 1995)

Some researchers also reported that fermentation can affect sensory properties such as taste, color, and certain physical properties, such as viscographic characteristics (Hounhouigan, Mjr, Nago, Houben, & Rombouts, 1993;

Namugumya & Muyanja, 2009). Due to these properties, fermentation can improve the shelf life, food safety, and acceptability of maize-based foods. Considering the possible inefficiency of spending several hours on fermentation, some industries choose not to ferment the maize or to ferment only a few hours. Therefore, in this study, unfermented porridge and fermented porridge will both be cooked and the carotenoid content and bioaccessibility of carotenoid compared.

2.5 Carotenoid characteristics and carotenoids in maize

Carotenoids are natural pigments which play various biological roles in plants and animals. Until now, more than 600 carotenoids have been identified. They are composed of two species of hydroxylated xanthophylls that carry at least one oxygen atom (such as lutein, zeaxanthin, and α-cryptoxanthin) and carotenes which only contain carbon and hydrogen (such as α- and β-carotene, and lycopene) (Kean et al. 2011). Carotenoids provide some of the yellow, orange, and red color in fruits, flowers, and vegetables. Carotenoids can only be synthesized by plants, bacteria, fungi, and algae, and plants provide the main sources of carotenoids in most human diets. Forty different carotenoids are consumed by humans, and 20 of these are present in human blood and tissues. Major carotenoids in human diets are β-carotene, α-carotene, lycopene, lutein, and cryptoxanthin, which constitutes almost 90% of the total carotenoid consumption (Rao & Rao, 2007). Different carotenoids appear in different dietary sources, such as α-carotene and β-carotene, which are found in yellow/orange or dark green vegetables and fruits like carrots and broccoli. Lutein and zeaxanthin are present in dark, green vegetables, while lycopene is found in tomatoes.

The polyisoprenoid structure is the basic structure for all carotenoids. It consists of a long-conjugated chain of double bonds as backbone and symmetrical groups at the two ends of the chain. Figure 2 shows several common carotenoids with this polyisoprenoid structure (Rao & Rao, 2007). In nature, carotenoids are generally present as all-trans form. In dietary foods, low levels of trans carotenoids can isomerize to form cis-trans configuration due to the long conjugated double bonds structure (Stahl & Sies, 2003).

Carotenoids are among the phytochemicals which contribute to beneficial health effects because of their functional properties. Usually, plants with yellow-orange or dark green color are rich in carotenoids (Britton et al. 2009). For humans, the most familiar characteristic of some carotenoids, called provitamin A carotenoids, is the enzymatic conversion to vitamin A. In food, β-carotene is mostly distributed and regarded as the most valuable provitamin A carotenoid. After the enzymatic reaction by β-carotene monooxygenase, β-carotene is broken into two retinal molecules and then converted to vitamin A. Besides, α-carotene and β-cryptoxanthin are also provitamin A carotenoids, but they only have half the activity of β-carotene (Tanumihardjo, 2002)

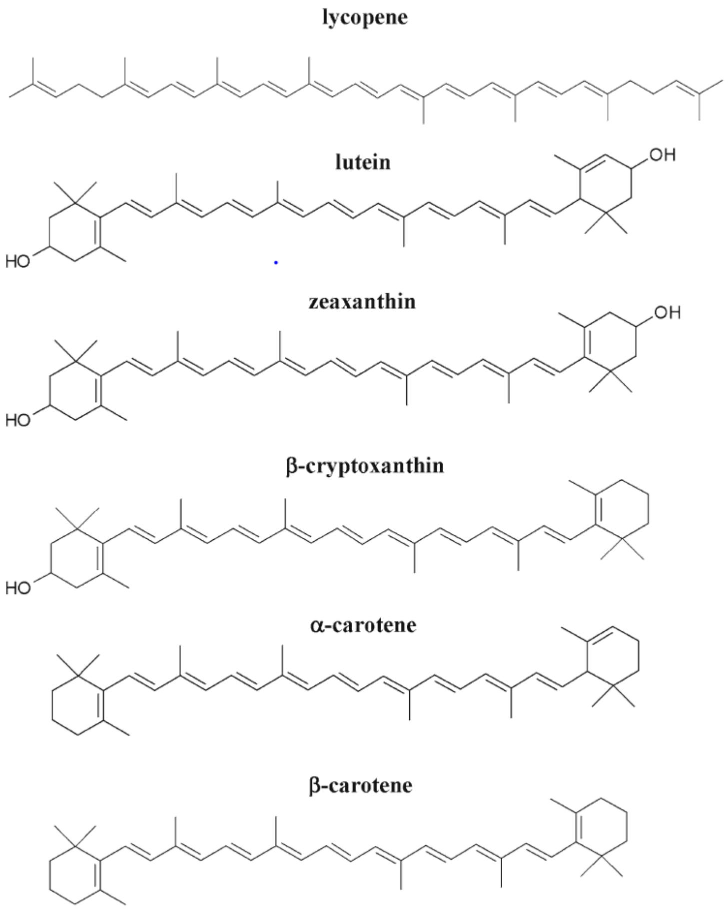


Figure 2.1. Structures of carotenoids commonly found in foods (Tanumihardjo, 2002)

For maize and maize products, the predominant carotenoids are lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene, in that order (Thakkar et al., 2007). Generally, yellow and purple maize contain more carotenoids, especially in lutein and zeaxanthin content, than white ones (de la Parra et al., 2007). Based on Ndolo and Beta's (2013) report, the carotenoids, lutein, and zeaxanthin are concentrated in the aleurone layer and endosperm of the maize kernel, which is different from other non-corn cereal. According to Harjes et al. (2008), carotenoids of some maize genotypes can reach as high as 66.0 μg/g. Also, from previous work, among orange maize in Dedza, central Malawi, carotenoid content can range from 50.8 to 72.6 µg/g. Lutein and zeaxanthin are major carotenoids which constitute almost 70% of total carotenoids in maize and maize products (Thakkar et al., 2007; Sesso et al., 2004). As for provitamin A carotenoids in maize, according to Kurilich and Juvik (1999), they constitute only 2% to 10% of total carotenoids.

2.5.1 Carotenoids absorption and transportation

After release from the food matrix, carotenoids are mixed into micelles which are incorporated with bile acids and dietary fat before they are transferred as chylomicrons in the small intestinal system. Carotenoids as lipophilic compounds usually cooperate with the lipid phase from which they are absorbed by the small intestinal mucosa through diffusion and then packaged into triacylglycerol-rich chylomicrons. Xanthophylls such as lutein and zeaxanthin are cleaved before absorption by the small intestinal mucosa and provitamin A carotenoids, such as β-carotene, are converted to vitamin A partially in chylomicrons and then finally transferred to the liver. Lipoproteins serve as carriers during transportation of carotenoids in plasma.

2.5.2 Health benefits of carotenoids

Vitamin A deficiency (VAD) is a severe public health problem throughout the world, especially in low-income developing countries. Vitamin A and its metabolites are essential for vision, the immune system, and the development of an embryo (Bone et al., 1997). VAD can lead to high disease and mortality rates, especially in pregnant woman and preschool-age children, because in these periods the body will present more nutritional demands than during other periods of development.

Chronic low vitamin A diet is one of the leading causes of VAD (Salud & UNICEF, 2005). In some developing countries, people must obtain their dietary vitamin A from small amount of plant and vegetable provitamin A carotenoids. In addition to being able to help with vitamin A deficiency, provitamin A carotenoid can also help prevent or reduce incidence of some cancers. Some studies have shown their benefits in reducing or preventing chronic diseases, such as lung cancer and prostate cancer (Kim et al., 2006). Unlike β-carotene, β-cryptoxanthin, and α-carotene, lutein and zeaxanthin do not have provitamin A properties. These two carotenoids can contribute to age-related macular degeneration (AMD), which is associated with people's retinas (Schalch et al., 2007).

2.5.3 Effects of processing on carotenoids

Since carotenoids are susceptible to oxidation and isomerization, food processing and cooking can result in some quantitative and qualitative changes. Also, during processing, disruption of the matrix of food material may lead to bioaccessibility and changes in some functional properties. Different cooking and processing methods will lead to different carotenoid content loss. For example, cooking longer or at higher temperatures can lead to higher loss, while stir frying and microwaving can lead to less loss (Britton et al., 2009). The main cause of carotenoid loss is oxidation, but isomerization also occurs during processing. Although isomerization will almost certainly not change total carotenoid content, it will change the biological activity due to an increase in Z isomers by up to 40%. According to Li et al. (2007) and Rodriguez-Amaya (1997), in eastern and southern African countries, maize is mostly cooked by adding maize flour to boiling water to form porridge. Before cooking, raw maize kernels usually go through the soaking and ‘wet milling’ step. During soaking and ‘wet milling,’ provitamin A carotenoids will be lost at a gross less than 10%. After milling to flour, some peoples will ferment the maize flour, especially in some African countries. Based on data from Li et al. (2007), the retention rate of provitamin A carotenoid after ‘wet-milling’/fermenting/boiling was 72.5%, which is slightly lower than wet-milling/unfermented/boiling (75%). In the study of Kean et al. (2008), researchers observed the carotenoid retention and bioaccessibility of three maize-based products (puff, bread, and porridge) using regular and whole yellow corn meal. According to their results, they found an average retention of about 62% for total carotenoids among all three food products, including 75% retention for bread and 53% for porridge.

2.6 Bioavailability and bioaccessibility of carotenoid

Even though carotenoids have several health benefits, they have to be absorbed by people or animals before they can be efficient in beneficial health roles. Carotenoids absorption is quite complex. First, carotenoid-containing food products must pass through the oral system. Carotenoids will be released during enzymatic reaction. Then, carotenoids combined with co-consumed oil form chyme in the stomach and proceed into the small intestine. Then, in the intestinal tract, bile will be released from the gall bladder and triacylglycerides will be broken down into mono-, diglycerides, and fatty acids via an enzymatic reaction; these resultant products will combine with bile and form micelles. Carotenoids will dissolve in micelles (Furr & Clark 1997; Nagao 2009). Some carotenoids will then be taken by the intestinal epithelial cells and lastly transferred to lymphatic system as chylomicrons (And & Russell, 2002; Yonekura & Nagao, 2010). Bioavailability can be used to describe this whole process. Bioavailability is defined as the fraction of carotenoids from a food meal that can be absorbed by tissue and utilized. In-vivo assay should be used to measure the bioavailability of carotenoid. However, among individual responses, there are huge variations which render the results nonrepresentative (Rodriguez-Amaya, 2015). Bioaccessibility is defined as the proportion of carotenoids from a food source that can be absorbed by intestinal epithelia. Before absorption by the intestinal epithelia cells, carotenoids are dissolved in micelles. Micellarization efficiency is used to measure carotenoids bioaccessibility and may also be used to estimate the bioavailability of carotenoids (Reboul et al., 2006). Some researchers have shown that carotenoids bioaccessibility can vary based on several factors, such as different genotypes (Li, Tayie, Young, Rocheford, & White, 2007), type of food source (Kean et al., 2011), cooking process (Thakkar et al., 2007), and addition of oil (O'Connell et al., 2008).

2.7 In-vitro assessment of antioxidant activity of carotenoid

In the early 1980s, β-carotene was found to be an antioxidant and to possess the ability to help reduce human cancer rates. Since then, researchers started to focus on carotenoids. Currently, many assays have been developed to measure antioxidant activity. Among these assays, ORAC, DPPH, TEAC/ABTS, and FRAP are usually used for food (Rodriguez-Amaya, 2015). In oxygen radical absorbance capacity (ORAC) and Trolox equivalence antioxidant capacity (TEAC/ABTS) assays, the antioxidant capacity is obtained by monitoring the decay of fluorescence (Ou et al., 2002). In the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, change of absorbance under UV/Vis is measured to represent the antioxidant activity when antioxidants react with DPPH radical (Adom & Liu, 2002). However, some researchers have questioned the efficacy of these assays since they are performed in aqueous and organic solvent environments. Studies also showed that using antioxidant activity determined by simple chemical assay to claim the health benefit of food is ineffective (Becker, Nissen, & Skibsted, 2004). During carotenoid digestion, some biologically active compounds will be formed. These compounds have some unique structural characteristics, including polyunsaturated double bonds and eight isoprenoid units which enable carotenoids to quench singlet oxygen and transfer electrons during oxidation and reduction reactions (Tanumihardjo, 2013).

2.8 Gap of knowledge

Several studies have shown the difference of carotenoid content between raw maize and maize-based products to figure out the effect of certain procedures during cooking (Li et al., 2007). Some studies also reported a carotenoid content variation after in-vitro or in-vivo digestion to express the bioavailability of carotenoid in different food products (Hotz & Gibson, 2007; Kean et al., 2011; Reboul et al., 2006). However, the data obtained are difficult to compare because the bioaccessibility and antioxidant capacity of carotenoids can be influenced by several factors, such as different genotype (Li et al., 2007), type of food source (Kean et al. 2011), cooking process (Thakkar et al., 2007) and addition of oil (O’Connell et al. 2008). Moreover, there are few studies reporting on the difference in cooking and other treatments among maize containing different carotenoid levels. In this case, it is worth using a well-structured set of maize samples to study the retention rate of carotenoid during processing and the bioaccessibility and antioxidant capacity of carotenoid after digestion.

Since carotenoids are fat soluble compounds, several studies have shown the interactions between carotenoid and lipid including the carotenoid’s solubility in lipid bilayer membranes (Xia et al., 2015; Popova & Andreeva, 2013; Salvia-Trujillo et al., 2013). Recently, some studies also reported the interaction between carotenoids and carotenoid-binding proteins to further study the transportation of carotenoids after digestion (Lafountain, Prum, & Frank, 2015; Vachali, Li, Bartschi, & Bernstein, 2015). Some studies also reported the retention rate of carotenoids during traditional porridge cooking in high β-carotene maize without comparing to low or medium β-carotene maize (Li et al., 2007).

3. DETERMINATION OF CAROTENOIDS AND ANTIOXIDANT CAPACITY FOLLOWING DIFFERENT PROCESSING TREATMENTS OF MAIZE

3.1 Abstract

Maize is one of the most consumed staple foods, containing various macro- and micronutrients. Carotenoids are important bioactive compounds with lutein and zeaxanthin occupying almost 70% of the total content in maize. In this study, carotenoid profiles, oxygen radical absorbance capacity (ORAC), and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity as antioxidant capacities of maize were determined for five maize-based products (boiled maize, roasted maize, fermented maize, fermented porridge, and unfermented porridge). Each treatment showed different levels of carotenoids retention. On average, boiled maize had the highest retention rate (79.0%), followed by fermented porridge (73.8%), fermented maize (68.4%), unfermented porridge (59.0%), and roasted maize with the lowest retention rate (56.1%). In conclusion, high temperature treatments such as roasting results in relatively high carotenoids reduction compared to low temperature treatments, and the fermentation step can prevent drastic reduction in carotenoids.

3.2 Introduction

Carotenoids are a group of plant pigments which have been proven to exhibit health benefits. During plant biosynthesis, carotenoids can prevent photo-oxidative reactions, take part in light harvesting process, and attract insects and birds with their color. In the human diet, carotenoids, including β-carotene, α-carotene and β-cryptoxanthin, are the predominant sources of vitamin A. Lutein and zeaxanthin intake can also benefit the human vision system.

According to The World Health Report, vitamin A deficiency has become the leading cause of acquired blindness in children and impaired vision in areas of several developing countries (Mutangadura, 2004). Vitamin A deficiency affects approximately 127 million preschool children and 7.2 million pregnant women (West, 2002). Carotenoids have been linked to the prevention of vitamin A deficiency (VAD), some skin problems, night blindness, and cancer. Due to these health benefits, the sources and quantity of carotenoids have become a topic of great interest to researchers.

Throughout many African countries, maize serves as a main staple food. According to the World Health Organization, in Africa, the average daily maize consumption is 106.2 g/ person, which occupies almost half of the average daily total cereal consumption (McCann, 2005). Porridge is a major traditional food cooked using maize. According to the National Food Consumption Survey (NFCS), more than 90% of South African children between 1 and 9 years old consume maize porridge (Khumalo, Schönfeldt, & Vermeulen, 2011).

Some studies also focus on the development of biofortified maize to enrich carotenoids and provitamin A contents. Muzhingi et al. (2008) determined major carotenoids (lutein, zeaxanthin α- and β-cryptoxanthin, and β-carotene) in 36 genotypes of yellow maize and investigated carotenoid changes after boiling and baking (Muzhingi et al., 2008). In another study on provitamin A biofortified maize, the retention of β-carotene was 75.5% and 75.2% for African traditional fermented and unfermented porridge, respectively (Li et al., 2007), and 64% for nixtamalization and frying during the preparation of Mexican-inspired products (Lozano-Alejo, Carrillo, Pixley, & Palacios-Rojas, 2007). These studies showed that cooking methods have a significant effect on retention of carotenoids in maize based products.

In this study, maize samples were acquired under the Malawi Farmer-to-Farmer Agroecological (MAFFA) project implemented from 2012 to 2018. To improve maize nutrition, especially for provitamin A content, this investigation was aimed at examining the effects of different traditional processing methods and cooking methods for fermented and unfermented porridge on carotenoid content and composition.

3.3 Materials and Methods

3.3.1 Chemical and Standards

HPLC grade hexane, methyl-butyl ether (MtBE), 1-butanol, and methanol were acquired from Fisher Scientific (Whitby, ON, Canada). Carotenoid standards, lutein (≥97.0%), zeaxanthin (≥97.0%), β-cryptoxanthin (≥97%), α-carotene (≥95%) and β-carotene (≥95%), 2,2′azobis (2-amidinopropane) dihydrochloride (AAPH) and 2,2,-diphenyl-1-picrylhydrazyl (DPPH) (95%), and butylated hydroxytoluene (BHT) were acquired from Sigma-Aldrich (St. Louis, MO, USA) and kept under -40^{o}C prior to analysis. Fluorescein and trolox (6-hydroxy-2,5,7,8-tramethylchroman-2- carboxylic acid) were acquired from Fisher Acros Organics (Morris Plains, NJ, USA). Deionized water (Mill-Q) was used in HPLC analysis.

3.3.2 Sample description

The maize samples were harvested in 2014 from central and northern regions of Malawi, respectively in the Lobi and Mzimba districts. The landrace orange maize seeds were one variety cultivated in Malawi, named Mthikinya (MW5021). The maize was shipped from Malawi to the University of Manitoba, sealed in clear plastic sample bags, and kept under -40^{o}C prior to analysis. In this study, twelve orange maize samples were selected based on their total carotenoids content. The 12 samples were further divided into three groups based on their genetic diversity shown in **Appendix I**, which are Group 1 including samples 07 and 08, Group 2 including samples 01, 04, 06, 09, 10, 11, and 12, and Group 3 including samples 02, 03, and 05.

3.3.3 Sample preparation

Raw maize kernels were processed as described below in sections 3.3.3.1 to 3.3.3.5. A portion of unprocessed kernels were retained and ground to a fine powder using a grinding mill (Micro-Mill, Bel-Art Products Co., Wayne, NJ) and a multi-use blade grinder (model PCC770, Loblaws Inc, Toronto, ON, Canada) to pass through a 0.5 mm sieve screen and then stored at -40^{o}C prior to analysis.

3.3.3.1 Boiling procedure

Maize kernels (10g) were boiled in distilled water (kernel:water ratio, 1:5) using a water bath set at a temperature of 95 ^{o}C for 1 hour. Cooked kernels were then dried in an air oven overnight at 55 ^{o}C. Boiled maize kernels were ground to pass through a 0.5 mm sieve screen using the same method as unprocessed maize samples and then stored at -40 ^{o}C before analysis.

3.3.3.2 Roast procedure

Maize kernels (10g) were roasted at 400^{o}C for 20 min and then ground to pass through a 0.5 mm sieve screen using the same method as unprocessed samples and then stored at -40^{o}C before analysis.

Images showing maize before roasting and after roasting

3.3.3.3 Fermentation procedure

The method used for preparation of fermented porridge was modified from Li et al. (2007). The maize was soaked in tap water (1:1 by weight) at room temperature in the dark for 24h. The samples were air dried overnight and milled using a grinding mill to pass through a 0.5 mm sieve screen.

The milled flour samples (55 g each) were mixed with 100 g of water. The mixture was allowed to ferment at room temperature for 48 hours while being protected from the light. Following this step, half of the fermented samples were freeze-dried and stored at -40^{o}C before analysis. During fermentation, no enzymes were added, and this was referred to as solid state fermentation.

3.3.3.4 Fermented porridge preparation

The procedure for fermented porridge preparation was modified from Li et al. (2007). A cold-boiling-water procedure was used to cook the porridge. Flour (50 g) and cold water (50 g) were mixed together to form a slurry. Then, boiling water (100 g) was poured into the slurry. The whole contents of this thin slurry were cooked for 9 min at 93^{o}C to obtain fermented porridge.

3.3.3.5 Unfermented porridge preparation

The unfermented porridge was prepared using the same stages as outlined for fermented porridge but without the fermentation step. After cooking, the porridge was cooled at room temperature for 1 hour. The samples were then freeze dried, milled using a grinding mill to pass through a 0.5 mm sieve screen, and then stored at -40^{o}C before analysis.

3.3.4 Proximate analysis of maize samples

3.3.4.1 One hundred-kernel weight

One hundred kernels were randomly obtained from each sample, counted, and weighed to get a one hundred-kernel weight (M. Blandino et al., 2010).

3.3.4.2 Endosperm hardness

Ten samples were randomly selected for each sample and vertically halved. Hardness was obtained by a subjective rating of 1-5 (1 implies 100% floury and 5 implies 100% vitreous) following measurement of the area of the total cut surface and that of the floury endosperm region (M. Blandino et al., 2010).

3.3.4.3 Moisture content determination

Moisture in raw and processed samples was determined according to the AACC International method 44-15.02 (AACC International, 1999).

3.3.4.4 Protein content determination

Protein content determination was done based on nitrogen combustion (TruSpect®. LECO Corporation, MI, USA) using approximately 10-20 mg of ground maize. The protein content of the maize samples was then calculated using the following equation: Protein content (%) = nitrogen content (%) × conversion factor of 6.25.

Protein determinations were done in duplicate.

3.3.4.5 Crude fat content determination

Crude fat content was determined using the Soxhlet apparatus (Lab-line Instruments, Inc., USA). A 250 mL flat bottom flask containing 3-5 boiling chips was pre-dried in an air-oven at 125 ℃ for 30 min and transferred into a desiccator to cool prior to weighing. Approximately 3 g of the sample were accurately weighed into an extraction thimble. A wad of glass wool was placed over the sample to prevent the sample loss. The thimble was then placed inside the Soxhlet unit. Hexane (160 mL) was added to the weighed 250 mL flat bottom flask. Samples were extracted for 16 h. After the extraction, hexane in the flask was evaporated using a heating mantle in the fume hood leaving the crude fat extracted from the sample in the flask. To ensure complete evaporation of the hexane, flasks with fat were placed in the air-oven at 100 ℃ for 1 h. After cooling to room temperature in the desiccator, flasks with fat were weighed. The calculation for crude fat content was as follows:

$$\%\ \text{crude fat} = \frac{\text{Weight after drying-weight of flask}}{\text{Weight of sample}} \times 100$$

The fat content determinations were conducted in duplicates.

3.3.4.6 Ash content determination

Total ash content of whole maize flour was determined according to the AACC International method 08-01.01 (AACC International, 1999).

3.3.5 Carotenoids extraction

Carotenoids were extracted according to the AACC method 14-60.1 (2012) with some modifications. Water-saturated butanol (WSB) was used as an extracting solvent. Using 15ml centrifuge tubes as vials for samples and reagents, 450-550mg of representative samples were mixed with 5 mL WSB in the fume hood. The mixture was homogenized, and carotenoids were extracted twice by shaking 15 min and settling 60 minutes at room temperature. Then, the tubes were centrifuged for 5 minutes at 4,000 g at 20^{o}C. The supernatant was transferred to another amber centrifuge tube prior to analysis.

3.3.6 HPLC analysis of carotenoids content

Identification and quantification of carotenoids were carried out using HPLC (Waters 2695, Milford, MA) equipped with a photodiode array (PDA) detector (Waters 996, Milford, MA) and an autosampler (Waters 717 plus, Milford, MA) (Hwang et al., 2016). The column was operated at 35^{o}C and eluted with a gradient mobile system consisting of (A) methyl tert-butyl ether and (B) 1% water in methanol at 1 mL/min. The gradient was programmed as follows: 0–5 min, 5–10% B; 15–16 min, 10–20% B; 21–22 min, 20–30% B; 29–30 min, 30–38% B; 36–45 min, 100% B; 46–50 min, 5% B (Hwang et al., 2016). The separated carotenoids were detected and measured at 450 nm. Composition of carotenoids in samples was determined by a comparison of peaks to the retention time of carotenoid standard peaks and their maximum UV/Vis absorption in the chromatograms obtained. The HPLC analyses were carried out in duplicate.

3.3.7 Oxygen radical absorbance capacity assay (ORAC)

The antioxidant activity was determined according to the ORAC assay described by Huang et al. (2002), with some modifications. The assay made use of a 96-well flat bottom polystyrene. A Precision 2000 well automated microplate pipetting system (Bio-Tek Instruments, Inc., Winooski, VT, USA) was used to transfer 150 μL fluorescein with 25 μL trolox standard or extracts into each well. The microplate was shaken in the microplate reader for 3 min, and then incubated at 37°C for another 15 min. Then, 25 μL AAPH was transferred to each well and the fluorescence intensity was determined automatically every minute for 50 min by a FLx800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) with an excitation wavelength of 485±20 nm and an emission wavelength of 528±20 nm. The final results were expressed as a Trolox equivalent and the antioxidant capacity was determined based on the Trolox standard curve. Trolox standards of 12.5, 25, 50, and 100 μM were used. Antioxidant capacity was calculated based on the method by Huang et al. (2016). Regression equation between Trolox concentration and the net area under the fluorescence decay curve was constructed. The analysis was conducted in triplicates. Relative ORAC Value = $\frac{AUC_{sample}-AUC_{blank}}{AUC_{Trolox}-AUC_{blank}}(\frac{Molarity\ of\ trolox}{Molarity\ of\ sample})$, where AUC_{sample}, $AUC_{blank,}$ and AUC_{Trolox} were the areas under curve of sample, blank, and Trolox. The formula of area under curve was as follows: AUC$= 0.5+\frac{f_1}{f_0}+\cdots+\frac{f_i}{f_0}+\cdots+\frac{f_{49}}{f_0}+0.5\frac{f_{50}}{f_0}$, where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at i min.

3.3.8 DPPH radical scavenging activity assay

The DPPH method was used according to the modified method used by Beta et al. (2005). A 60 µmol/L DPPH• reactant was made in methanol. Then, 190 µL of a DPPH• solution was added to 10 µL of the sample and the absorbance at 515 nm was measured at t = 30 min. To determine the absorbance at t = 0 min, measurement was taken by adding 190 µL of the DPPH• solution to 10 µL of methanol. The antioxidant activity was calculated as % DPPH• scavenging activity = (1 - [$A_{sample,t=30}$ / $A_{control,t=0}$]) × 100, where $A_{control}$ is the absorbance of DPPH radical in methanol at 0 min, and A_{sample} is the absorbance of DPPH radical for the sample extract or standard at 30 min. Trolox standards of 12.5, 25, 50, and 100 µM were used. A calibration curve of % DPPH decolorization obtained from different concentration of trolox was used to quantify the antioxidant capacity of the extracts. Antioxidant capacity was expressed using Trolox equivalents (TE) per g of dry samples. The analysis was conducted in triplicates.

3.3.9 Statistical analysis

Results were reported as means ± standard deviation (SD) of triplicate determinations. Analysis of variance (ANOVA) for the main factor (processing methods) was determined by using a GLM procedure with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Variance was considered significant when $p <0.05$ using a Tukey-Kramer test. In addition, Pearson correlation tests were used to evaluate the correlation among variables at significant levels of $p < 0.05$.

3.4 Results and Discussion

3.4.1 Physicochemical properties of traditional maize varieties

Means and standard deviations of compositional and physical properties are presented in Table 3.1. Determination of maize kernel quality and substantive nutritional information can help with comprehending overall characteristics of maize (Nago, Akissoë, Matencio, & Mestres, 1997).

Moisture is one of the most important parameters of grain quality. Moisture content is related to dry matter content in kernels and can therefore affect the yield and price of the grain. Moisture is also related to post-harvest control and storage. The higher moisture content, the grain will be less stable during storage (Lee et al., 2007; Serna-Saldivar, 2016). Among twelve samples, moisture contents ranged from 12.10% to 16.01%, which confirmed previous studies by Hwang et al. (2016) who reported values of 11.21% to 17.77% of traditional maize varieties from Malawi.

Ash, crude fat, and crude protein ranged from 1.21% to 1.65%, 4.17% to 6.11%, and 5.45% to 9.91%, respectively, on a dry weight basis. The results are in accordance with Hwang et al. (2016). In her study, ash, crude fat, and crude protein in the maize sample from Malawi ranged from 1.07% to 1.96%, 4.24% to 7.23%, and 7.73% to 10.89%. There was no significant difference shown in crude fat and ash content. The slight differences between samples were likely due to environmental conditions before harvest and genetic change during open pollination.

The one hundred-kernel weight of three carotenoids-level maize ranged from 32.88 g to 40.67 g, which is in agreement with a previous reported average value of 38.90 g of the one hundred-kernel weight of orange maize (Hwang et al., 2016). The one hundred kernel weight or 1000 kernel weight is commonly used as a physical factor of maize and other cereals. It can indicate kernel size which in turn affects drying rates for volume-to-surface ratio.

Grain hardness is also a physical factor for grain quality control; it can be affected by the ratio of corneous to floury endosperm. In our method, maize kernels were halved to subjectively determine the ratio of corneous to floury endosperms, as Figure 1 depicts. Grain hardness was obtained by a subjective rating of 1-5, 1 implying 100% floury and 5 implying 100% vitreous. Higher numbers corresponded to harder endosperm. In the grain industry, softer grains can produce finer flours, and the resultant particle size distribution is useful for grading wheat by the USDA (Serna-Saldivar, 2016).

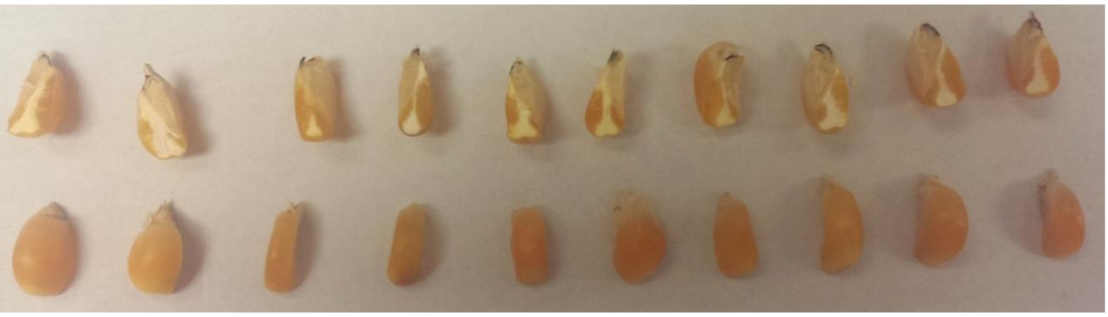
Table 3.1 Compositional and physical properties among samples

	Moisture %	Lipid%(dw)	Ash%(dw)	Protein%(dw)	100 seeds weight (g)	Endosperm hardness (rate 1-5)
Sample 1	13.25±0.02	4.51±0.15	1.21±0.01	7.64±0.19	34.63±0.45	3.25±0.61
Sample 2	12.10±0.01	4.70±0.02	1.51±0.06	7.54±0.01	38.83±0.37	3.85±-0.63
Sample 3	13.19±0.02	4.79±0.22	1.24±0.02	6.00±0.09	40.59±0.51	3.20±0.95
Sample 4	13.72±0.05	5.03±0.21	1.29±0.01	5.45±0.10	38.10±0.24	3.40±0.61
Sample 5	13.91±0.06	4.20±0.07	1.41±0.03	7.49±0.14	40.67±0.58	3.40±0.74
Sample 6	13.68±0.03	6.11±0.53	1.29±0.04	8.37±0.21	40.30±0.58	3.50±0.62
Sample 7	12.14±0.04	4.19±0.23	1.43±0.02	7.51±0.00	38.64±0.16	3.80±0.54
Sample 8	16.01±0.04	4.17±0.08	1.30±0.04	9.18±0.26	39.84±1.09	3.45±0.28
Sample 9	13.20±0.03	5.39±0.20	1.53±0.03	8.09±0.03	36.00±1.22	3.55±0.80
Sample 10	15.19±0.05	5.57±0.09	1.42±0.01	9.45±0.20	32.88±0.57	3.25±0.59
Sample 11	13.01±0.03	4.96±0.02	1.65±0.01	9.91±0.20	39.36±0.37	3.40±0.66
Sample 12	14.57±0.04	5.40±0.04	1.29±0.03	9.91±0.08	37.50±0.49	3.70±0.67

Hardness was obtained by a subjective rating between 1-5, 1 meaning 100% floury and 5 meaning 100% vitreous.

Higher numbers correspond to harder endosperm.

Figure 3.1 Grain hardness determination after cutting the kernel



3.4.2 Effect of processing treatments on carotenoids content

In this study, samples with different carotenoid contents were selected in order to evaluate the extent to which treatment could alter carotenoid content among foods produced by farmers growing maize in different environments. Carotenoid contents of unprocessed maize and maize-based products, including boiled maize, roasted maize, fermented maize, fermented porridge, and unfermented porridge, are shown in Table 3.2. Among these samples, lutein, zeaxanthin and β-cryptoxanthin, α-carotene, and β-carotene contents were analyzed using HPLC. A representative chromatogram of the carotenoid standard and an example of the sample chromatogram are shown in **Appendix II.**

Prior to treatment, individual carotenoids were analyzed by HPLC and the total carotenoids were calculated as a sum of the individual compounds. Lutein, zeaxanthin and β-cryptoxanthin, α-carotene, β-carotene, and total carotenoids ranged from 70.0 to 254.5, 36.6 to 104.6, 2.2 to 6.3, 1.6 to 4.3, 10.0 to 19.8, and 108.7 to 388.1 μg/g DW, respectively (Table 3.2a). Significant differences occured among these samples. Samples 1 to 12 were sorted according to total carotenoids content from lowest to highest. Based on Hwang's (2016) research, location was the main factor influencing the carotenoids' composition. The maize seeds were open pollinated, and cross-pollination could have occurred, as shown by the genetic analysis completed in the previous chapter, which could also contribute to differences in carotenoid composition.

Using boiling as one treatment, maize kernels were boiled whole. After boiling, lutein, zeaxanthin and β-cryptoxanthin, α-carotene, β-carotene, and total carotenoids ranged from 86.0 to 189.9, 16.6 to 72.9, 1.7 to 4.9, 2.2 to 5.8, 5.0 to 12.7, and 113.9 to 284.8 μg/g DW, respectively (Table 3.2b). Compared with unprocessed samples, the total carotenoids of samples 1 and 5 were slightly increased by 4.8% and 6.0%, while the rest showed decrease. For the boiled maize, the retention rate did not show any significant difference. However, carotenoids content differed significantly among the samples. After boiling, carotenoids showed an average retention rate of 79%. Sample 12 contained the highest amount of carotenoids before boiling, and the carotenoids level was still significantly higher than the rest of samples. Similar findings were shown in other studies. The carotenoids content of boiled fresh and frozen corn were analyzed in a study by Song et al. (2013). After boiling, carotenoids of fresh corn had an increase of 1.1 to 1.8 times. However, carotenoids content showed a decline of 47-80% for frozen maize (Junpatiw, Lertrat, Lomthaisong, & Tangwongchai, 2013).

For roasted maize, lutein, zeaxanthin and β-cryptoxanthin, α-carotene, β-carotene, and total carotenoids showed a significant decline for all samples compared with boiling (Table 3.2c). Samples 1 and 5, which had a slight increase in carotenoids after boiling, also showed a lower decrease during the roasting process. Samples 9 to 12, which are genetically more similar, had a retention rate ranging from 47.0% to 61.5%. Both methods used whole kernels. However, the difference between these two methods was due to the temperature and moisture involved. For the roasting method, a much higher temperature was applied, and moisture in the final product was much lower compared to boiling the maize kernel. The high temperature could be the reason for lower retention of carotenoids in roasted maize.

Starch is a predominate compound in maize, and carotenoids can also be affected by changes in starch during different cooking methods. During boiling, starch granule swelling happens first. When the water temperature increases, starch starts unfolding.

Carotenoids in starch systems are relatively stable; however, after starch unfolds, carotenoids become exposed to oxidative changes. The carotenoids content would decrease as a result (Ball, 2005). Following roasting, starch granules are tightly embedded within the protein matrix and densely packed without water involvement. Starch and protein internal bonds are hard to disrupt when lacking water and heat. However, the temperature for roasting was much higher than for boiling. Higher temperatures provide more energy to break carotenoids, resulting in lower carotenoids content in roasted rather than boiled maize.

Lutein and zeaxanthin contents both decreased after treatments. There’s no significant difference between the retention rate of lutein and zeaxanthin as shown in **Appendix III**. Lutein and zeaxanthin are two predominant carotenoids that occur in maize, usually present in all-trans isomer. However, some other researchers detected cis isomers of lutein and zeaxanthin in thermal processed vegetables (Updike et al., 2003). In carotenoids, the long chain of conjugated double carbon bonds can be affected by light, heat, and acid degradations (Chen et al., 1994). For boiling and roasting processes, when heat was introduced, trans double bonds from lutein and zeaxanthin were more sensitive to geometric isomerization, and some converted to a cis configuration.

After solid state fermentation, carotenoids had an average retention rate of 53.9%, 54.3%, and 70.1% for lutein, zeaxanthin, and β-carotene, respectively (Table 3.2d).

Only sample 5 showed no decrease. Samples 2 and 3 within the same genetic group showed similar retention rates of 30.4% and 30.5%, respectively. The order of carotenoids levels of fermented maize showed similar trends as found with unprocessed maize, which meant samples containing higher carotenoids content still maintained higher levels after solid state fermentation. The values were lower than reported by Li et al. (2007) where lutein, zeaxanthin, and β-carotene had retention rates of 81.3%, 78.5%, and 89.8%, respectively. Compared to liquid state fermentation, in solid state fermentation, microorganisms are grown on solid substrate without free liquid. Several studies have shown a better production of enzymes, surfactants, and other value-added products in solid state fermentation, as well as lutein, zeaxanthin, and β-carotene (Buzzini, 2001; Lio & Wang, 2012). This fermentation step is an essential step to home-made fermented porridge, especially among some African households.

As shown in Table 3.2e, lutein, zeaxanthin and β-cryptoxanthin, α-carotene, β-carotene, and total carotenoids in fermented porridge ranged from 51.1 to 224.0, 36.0 to 89.6, 1.4 to 4.7, 2.7 to 3.9, 2.0 to 14.3, and 90.7 to 332.1 μg/g DW, respectively. Except for samples 2 and 3, the total carotenoids content of fermented porridge had a retention rate ranging from 74.0% to 90.1% with no significant difference. After heating the fermented maize slurry, carotenoids content showed an increase compared with fermented maize. The final retention of carotenoids was 72.9% and 77.5% for lutein and zeaxanthin, respectively. Similar values of 77.5% and

74.5% retention for lutein and zeaxanthin, respectively, were found for fermented porridges (Li et al., 2007).

Unfermented porridge as a staple food is commonly consumed as many as three times a day in many African, Latin American, and Asian families (De Moura, Miloff, & Boy, 2015). In unfermented porridge, lutein, zeaxanthin, β-carotene, and total carotenoids had average retention rates of 59.6%, 57.5%, 63.0%, and 59.0%, respectively (Table 3.2f). From Table 3.2f, samples 2, 7, 8, and 12 had similar retention ranging from 25.2% to 37.1%. Sample 1 had an increase of 5.5%, while the rest of the samples had retention ranging from 63.2% to 73.5%. Compared to fermented porridge, unfermented porridge had a higher decline. According to Li et al. (2007), high β-carotene maize (*Zea mays*) was used, and the same fermented and unfermented porridge process was applied as in this study. The retention rate of carotenoids in unfermented porridge was 75.2%, which was also lower than fermented porridge (Li et al., 2007)

Table 3.2a Carotenoids content of maize and maize-based products (μg/g DW) (Raw maize)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	70.0±0.9^{e}	46.2±2.8^{cd}	2.2±0.1^{d}	-	-	108.7±5.0^{d}
Sample 2	89.7±9.4^{e}	51.9±3.5^{cd}	3.3±1.7^{bcd}	3.2±0.0^{a}	10.5±4.9^{ab}	126.5±13.1^{d}
Sample 3	129.3±8.6^{e}	36.6±3.1^{d}	2.9±0.5^{cd}	-	12.0±2.0^{ab}	180.8±10.9^{cd}
Sample 4	142.7±7.5^{de}	37.6±3.04^{d}	3.2±1.1^{cd}	-	10.0±0.7^{ab}	193.5±8.3^{cd}
Sample 5	134.9±23.4^{de}	48.4±19.1^{cd}	3.2±1.4^{cd}	3.1±0.0^{a}	11.9±7.3^{ab}	199.8±53.4^{cd}
Sample 6	174.1±19.6^{cd}	58.6±19.43^{cd}	4.3±1.3^{bcd}	3.5±0.0^{a}	19.8±6.0^{a}	258.4±48.9^{cd}
Sample 7	196.0±27.7^{bc}	80.9±16.5^{bc}	4.9±0.7^{bcd}	1.6±2.0^{a}	17.6±0.0^{ab}	289.2±28.2^{c}
Sample 8	206.8±6.2^{abc}	75.4±3.0^{cd}	4.5±0.1^{bcd}	1.8±1.2^{a}	16.0±0.2^{ab}	299.2±11.0^{c}
Sample 9	241.0±4.6^{ab}	95.7±0.6^{ab}	5.3±0.1^{abc}	4.6±0.6^{a}	17.1±1.1^{ab}	363.5±4.9^{ab}
Sample 10	226.5±10.9^{ab}	104.6±7.0^{a}	5.4±0.2^{b}	3.7±0.3^{a}	19.8±1.6^{a}	360.1±19.8^{ab}
Sample 11	244.4±11.5^{a}	97.1±4.7^{ab}	5.6±0.5^{ab}	4.2±0.3^{a}	17.3±1.9^{ab}	368.6±18.7^{ab}
Sample 12	254.5±15.3^{a}	103.7±7.1^{a}	6.3±1.0^{a}	4.3±2.1^{a}	19.4±3.4^{a}	388.1±24.6^{a}

Table 3.2b Carotenoids content of maize and maize-based products (μg/g DW) (Boiled maize)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	86.0 ± 5.7^{g}	16.6 ± 1.0^{e}	1.7 ± 0.1^{f}	2.2 ± 0.2^{a}	8.1 ± 1.6^{b}	113.9 ± 9.3^{ef}
Sample 2	38.8 ± 10.4^{f}	19.6 ± 3.6^{de}	1.7 ± 0.2^{f}	2.5 ± 0.0^{a}	5.0 ± 1.4^{ab}	66.0 ± 16.5^{f}
Sample 3	103.3 ± 26.5^{f}	24.9 ± 5.6^{de}	2.4 ± 0.4^{de}	2.4 ± 0.0^{a}	6.2 ± 1.5^{ab}	137.6 ± 34.9^{e}
Sample 4	111.3 ± 4.8^{ef}	31.0 ± 1.6^{cd}	2.1 ± 0.1^{ef}	2.5 ± 0.0^{a}	10.0 ± 2.4^{ab}	155.2 ± 9.0^{de}
Sample 5	139.3 ± 2.2^{cd}	55.9 ± 1.5^{b}	3.9 ± 0.2^{abc}	2.2 ± 0.0^{a}	11.9 ± 2.0^{ab}	211.8 ± 4.8^{bc}
Sample 6	147.7 ± 8.0^{cd}	40.7 ± 2.4^{c}	3.3 ± 0.2^{cd}	-	11.3 ± 2.8^{b}	203.0 ± 13.0^{bcd}
Sample 7	165.0 ± 3.0^{bcd}	71.5 ± 1.2^{a}	4.5 ± 0.5^{ab}	2.8 ± 0.4^{a}	11.9 ± 0.3^{ab}	254.7 ± 4.4^{ab}
Sample 8	141.1 ± 19.8^{de}	42.5 ± 6.4^{c}	3.1 ± 0.4^{de}	3.5 ± 1.1^{a}	9.7 ± 4.1^{ab}	199.7 ± 31.0^{cd}
Sample 9	180.6 ± 10.1^{abc}	70.9 ± 4.9^{a}	4.9 ± 0.7^{a}	4.2 ± 0.2^{a}	12.6 ± 2.3^{a}	273.1 ± 18.1^{a}
Sample 10	154.1 ± 9.4^{bcd}	72.8 ± 4.9^{a}	3.7 ± 0.3^{abc}	2.9 ± 0.2^{a}	10.9 ± 1.9^{ab}	243.4 ± 15.6^{ab}
Sample 11	188.4 ± 11.1^{ab}	61.9 ± 6.4^{ab}	4.4 ± 0.4^{ab}	4.2 ± 0.0^{a}	12.7 ± 1.3^{ab}	271.5 ± 19.8^{a}
Sample 12	189.9 ± 6.2^{a}	72.9 ± 4.2^{a}	4.5 ± 0.6^{ab}	5.8 ± 3.8^{a}	11.6 ± 1.5^{a}	284.8 ± 14.8^{a}

Table 3.2c Carotenoids content of maize and maize-based products (μg/g DW) (Roasted maize)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	54.9 ±17.9^{ef}	15.2 ±5.5^{e}	1.8 ±0.3^{b}	-	4.5 ±1.3^{d}	76.5 ±24.8^{e}
Sample 2	33.9 ±8.1^{f}	21.4 ±2.3^{de}	1.8 ±0.1^{b}	-	6.9 ±0.9^{cd}	64.1 ±10.4^{e}
Sample 3	55.4 ±9.5^{ef}	32.4 ±3.9^{de}	2.7 ±0.4^{ab}	6.6 ±0.0^{a}	14.2 ±2.7^{a}	106.9 ±19.2^{de}
Sample 4	73.1 ±6.8^{de}	20.1 ±1.5^{e}	1.7 ±0.1^{b}	-	5.6 ±0.6^{d}	100.5 ±7.7^{de}
Sample 5	109.1 ±7.2^{bc}	33.7 ±2.7^{b}	2.7 ±0.3^{ab}	4.1 ±1.4^{ab}	10.9 ±2.0^{abc}	159.2 ±10.3^{bc}
Sample 6	95.8 ±13.3^{cd}	27.2 ±3.7^{ced}	2.8 ±0.3^{b}	-	6.4 ±0.8^{cd}	132.1 ±15.5^{cd}
Sample 7	75.3 ±3.9^{de}	39.4 ±2.4^{bc}	3.3 ±0.5^{a}	2.6 ±0.0^{b}	9.1 ±2.0^{bcd}	127.9 ±0.1^{cd}
Sample 8	78.8 ±10.6^{cde}	37.5 ±5.6^{bc}	2.8 ±0.6^{ab}	-	7.6 ±2.0^{bcd}	126.7 ±18.1^{cd}
Sample 9	152.3 ±12.9^{a}	54.0 ±3.8^{a}	3.5 ±0.6^{a}	4.2 ±1.2^{b}	11.0 ±2.0^{abc}	223.6 ±19.6^{a}
Sample 10	132.2 ±13.2^{ab}	56.5 ±6.1^{a}	2.8 ±0.1^{ab}	2.2 ±0.0^{b}	8.6 ±1.6^{bcd}	202.2 ±17.5^{ab}
Sample 11	147.7 ±3.2^{a}	55.7 ±0.9^{a}	3.5 ±0.3^{a}	3.1 ±0.3^{b}	10.7 ±1.6^{abc}	220.6 ±3.5^{a}
Sample 12	108.43 ±13.52^{bc}	55.17 ±6.54^{a}	3.69 ±0.52^{a}	2.95 ±0.60^{b}	12.13 ±1.57^{ab}	182.4 ±18.7^{ab}

Table 3.2d Carotenoids content of maize and maize-based products (µg/g DW) (Fermented Maize)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	49.7 ± 0.7^{g}	13.4 ± 1.4^{gh}	1.6 ± 0.1^{fg}	-	3.2 ± 0.2^{f}	67.8 ± 1.6^{g}
Sample 2	24.9 ± 2.4^{h}	11.4 ± 0.7^{h}	-	-	3.1 ± 0.5^{f}	38.4 ± 4.8^{h}
Sample 3	32.8 ± 4.9^{h}	17.3 ± 1.7^{g}	1.5 ± 0.1^{g}	-	3.4 ± 0.9^{f}	55.1 ± 6.1^{gh}
Sample 4	112.9 ± 3.8^{e}	36.5 ± 1.4^{f}	2.2 ± 0.1^{ef}	-	6.7 ± 3.1^{cde}	158.3 ± 5.6^{e}
Sample 5	138.9 ± 7.7^{d}	54.6 ± 2.5^{d}	3.3 ± 0.5^{bcd}	3.2 ± 1.0^{a}	8.7 ± 1.6^{cde}	208.7 ± 11.8^{d}
Sample 6	168.0 ± 5.4^{b}	52.2 ± 1.8^{de}	3.5 ± 0.2^{bc}	4.0 ± 0.7^{a}	10.9 ± 0.1^{abc}	238.6 ± 6.6^{c}
Sample 7	79.6 ± 4.7^{f}	39.3 ± 1.8^{f}	2.7 ± 0.0^{de}	-	4.8 ± 0.9^{ef}	126.5 ± 5.8^{f}
Sample 8	153.5 ± 4.3^{c}	48.7 ± 2.9^{e}	2.9 ± 0.3^{cd}	2.9 ± 0.7^{a}	8.8 ± 0.8^{bcd}	215.9 ± 9.8^{d}
Sample 9	168.8 ± 7.2^{b}	67.5 ± 2.5^{c}	3.3 ± 0.2^{bcd}	2.2 ± 0.0^{a}	7.3 ± 0.5^{bcd}	247.8 ± 8.7^{d}
Sample 10	173.1 ± 2.2^{b}	78.2 ± 1.9^{b}	3.4 ± 0.0^{bcd}	2.6 ± 0.6^{a}	11.6 ± 0.3^{ab}	268.9 ± 3.6^{b}
Sample 11	209.8 ± 4.9^{a}	86.9 ± 1.0^{a}	4.4 ± 0.1^{a}	4.4 ± 0.6^{a}	12.8 ± 1.4^{a}	318.4 ± 7.3^{a}
Sample 12	201.6 ± 2.4^{a}	65.3 ± 0.4^{c}	3.9 ± 0.1^{ab}	3.9 ± 0.7^{a}	10.1 ± 1.1^{abc}	284.9 ± 1.5^{b}

Table 3.2e Carotenoids content of maize and maize-based products (μg/g DW) (Fermented Porridge)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	51.1 ±4.2 ^{g}	36.0 ±1.0 ^{e}	1.6 ±0.0 ^{ef}	-	2.0 ±0.0 ^{d}	90.7 ±5.2 ^{d}
Sample 2	22.3 ±0.1^{f}	12.9 ±0.4^{cd}	1.4 ±0.1^{f}	-	2.6 ±0.0^{cd}	37.2 ±2.2^{e}
Sample 3	61.1 ±16.8^{f}	37.6 ±10.3^{e}	2.2 ±0.4^{def}	-	2.1 ±1.7^{d}	103.1 ±29.3^{d}
Sample 4	105.9 ±3.8^{e}	30.8 ±1.0^{d}	1.8 ±0.1^{ef}	-	4.5 ±0.6^{cd}	143.1 ±5.5^{d}
Sample 5	113.9 ±46.7^{de}	44.0 ±17.3^{cd}	2.8 ±0.9^{cde}	-	5.9 ±2.0^{c}	166.8 ±67.1^{cd}
Sample 6	155.4 ±4.3^{cd}	47.3 ±0.8^{cd}	3.2 ±0.3^{bc}	-	9.2 ±0.2^{b}	215.3 ±5.7^{bc}
Sample 7	156.8 ±5.0 ^{cd}	60.0 ±1.2 ^{b}	3.9 ±0.2 ^{c}	-	9.3 ±0.0 ^{b}	230.0 ±6.4 ^{bc}
Sample 8	153.20 ±0.0^{cd}	58.8 ±0.0^{bc}	3.3 ±0.0^{bcd}	-	12.4 ±0.0^{ab}	227.9 ±0.0^{bc}
Sample 9	183.7 ±2.0^{bc}	76.7 ±1.3^{ab}	3.4 ±0.2^{bc}	2.7 ±0.0^{a}	9.3 ±0.3^{b}	274.3 ±2.7^{ab}
Sample 10	166.4 ±1.2^{c}	74.7 ±0.8^{ab}	3.2 ±0.1^{bcd}	3.4 ±0.0^{a}	9.2 ±0.6^{b}	254.8 ±1.9^{b}
Sample 11	219.5 ±5.4^{ab}	89.6 ±2.2^{a}	4.7 ±0.0^{a}	3.9 ±1.2^{a}	14.3 ±0.3 ^{a}	332.1 ±8.3^{a}
Sample 12	224.0 ±0.4^{a}	77.0 ±0.9^{ab}	4.0 ±0.2^{ab}	3.9 ±0.2^{a}	12.8 ±0.5^{a}	321.7 ±1.7^{a}

Table 3.2f Carotenoids content of maize and maize-based products (μg/g DW) (Unfermented Porridge)

	lutein	Zeaxanthin	β-cryptoxanthin	α-carotene	β-carotene	Total*
Sample 1	81.1 ±1.3^{cd}	23.2 ±0.5^{d}	2.0 ±0.0^{cd}	3.7 ±0.0^{a}	4.7 ±1.3^{bcd}	114.6 ±2.2^{bc}
Sample 2	22.7 ±5.1^{f}	12.9 ±1.5^{e}	-	-	3.1 ±0.0^{d}	36.6 ±8.2^{d}
Sample 3	71.0 ±1.7^{de}	33.0 ±1.0^{cd}	2.0 ±0.1^{cd}	2.6 ±0.0^{a}	7.1 ±2.3^{bcd}	114.2 ±3.7^{bc}
Sample 4	107.3 ±3.3^{c}	25.7 ±0.9^{d}	2.0 ±0.2 ^{cd}	-	6.3 ±1.7^{cd}	141.4 ±4.7^{b}
Sample 5	104.0 ±33.1^{c}	38.5 ±10.5^{c}	2.8 ±0.4^{bc}	4.4 ±0.0^{a}	9.2 ±2.3^{bcd}	152.9 ±46.1^{b}
Sample 6	103.2 ±7.3 ^{c}	30.4 ±1.3 ^{d}	2.0 ±0.1 ^{cd}	-	6.2 ±2.1 ^{cd}	141.8 ±10.8 ^{b}
Sample 7	61.7 ±8.7^{de}	40.0 ±2.5^{c}	2.3 ±0.0^{bcd}	-	3.4 ±0.6^{d}	107.4 ±11.3^{bc}
Sample 8	46.8 ±5.0^{ef}	23.1 ±0.9	1.5 ±0.1^{d}	-	4.0 ±1.4^{d}	75.5 ±4.2^{cd}
Sample 9	181.6 ±2.6^{a}	62.6 ±1.2^{b}	4.0 ±0.3^{a}	3.7 ±0.62^{a}	11.0 ±0.5^{ab}	262.9 ±3.3^{a}
Sample 10	150.2 ±4.1^{b}	63.3 ±1.4^{b}	3.0 ±0.0^{b}	-	10.4 ±1.4^{abc}	226.9 ±5.6^{a}
Sample 11	175.1 ±2.0^{ab}	73.7 ±1.1^{a}	4.0 ±0.5 ^{a}	3.6 ±0.78^{a}	11.6 ±0.7^{a}	268.1 ±4.9^{a}
Sample 12	102.6 ±1.8^{c}	44.1 ±1.2^{c}	2.4 ±0.2^{bcd}	-	3.5 ±0.4	137.9 ±26.1^{b}

Values are means of three replicates ± SD.

- Not detected.

Mean values having the same letters within a column are not significantly different, $P < 0.05$

*Total Carotenoids = Lutein+ Zeaxanthin+ β –cryptoxanthin+ α-carotene+ β-carotene

In order to figure out which maize-based products could provide more carotenoids, Tukey-Kramer Grouping was performed using a SAS program based on the carotenoids content remaining in different products. Based on Tables 3.2b and 3.2d, and using lutein as an example, carotenoids levels were significantly higher for boiling than fermentation. Also, since the samples are genetically different, their performances during different treatments also showed differently. From Tables 3.2a to 3.2f, the 12 samples were not performing the same even though samples 9, 10, 11, and 12 were in the same genetic group, according to **Appendix I**. The percentage loss of lutein, zeaxanthin, and β-carotene after different treatments were similar. The ranking of the processed sample groups from highest to lowest was as follows: boiled maize, fermented porridge, fermented maize, unfermented porridge, and roasted maize. Sample 12 was used as an example to show changes in carotenoids following each treatment, as shown in Figure 3.2.

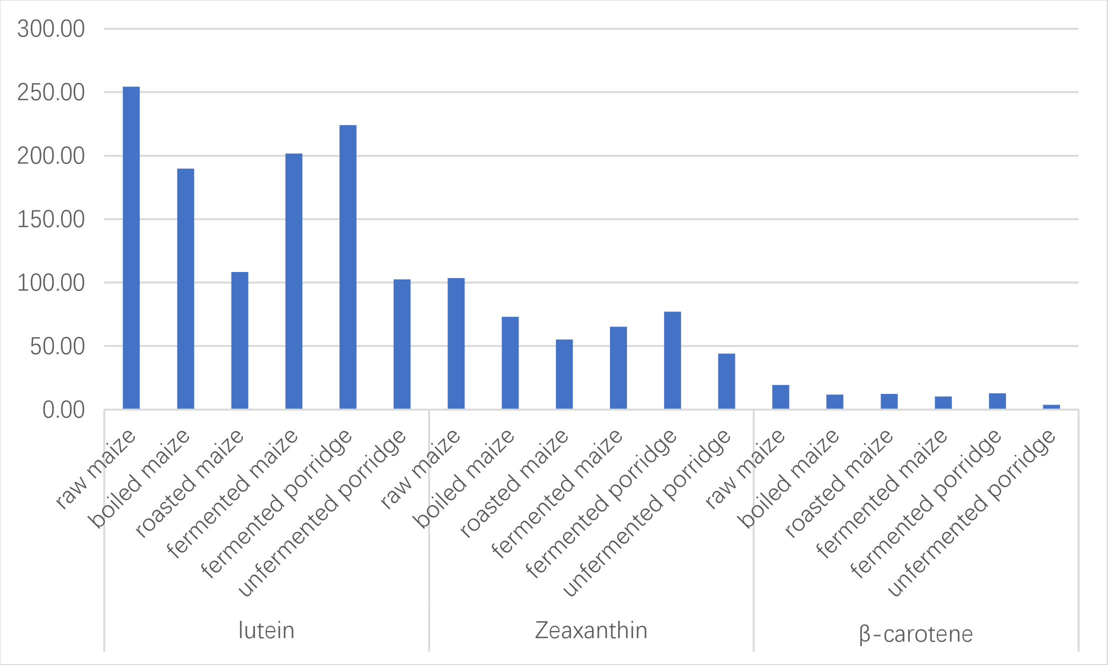
Comparing these five treatments, the most carotenoids remained after the boiling process. During boiling, maize is cooked in whole kernels, and the outer pericarp layers serve to prevent carotenoids releasing into the water. During fermentation, microorganisms from maize, such as *Lactobacillus fermentum* and *Lactocillus brevis*, grow, and pH could drop from 6.1 to 3.6 (Agume Ntso et al., 2017). A pH decrease can provide a better environment for growth of lactic acid bacteria. A pH drop can also stimulate phytate enzymatic degradation, which may increase the availability of certain minerals such as zinc, iron, and calcium. Carotenoids are likely released from the unfolding starch-protein matrix of the maize endosperm. When making porridge from unfermented maize flour and fermented maize flour using the same cooking method, fermented porridge had better retention of carotenoids than unfermented porridge. The fermentation step is the only difference between these two porridge-making processes. Roasting is the only method where water is not added, and the high temperature used in this process can cause degradation of the carotenoid structures. Therefore, the carotenoids content for roasted maize was the lowest among all five treatments. In this case, boiled maize had the highest carotenoids and therefore the highest provitamin A intake among populations consuming processed maize. When comparing fermented porridge and unfermented porridge as a common meal in African countries, fermented porridge would provide more provitamin A than unfermented porridge.

Table 3.3 Tukey-Kramer Grouping for treatments Least Squares Means (Alpha=0.05)

Maize-based Products	**Average carotenoids content for lutein (µg/g DW)**		
Boiled Maize	80.9966		A
Fermented Porridge	73.4689	B	A
Fermented Maize	69.0972	B	
Unfermented Porridge	62.5146	B	C
Roasted Maize	54.1200		C

Least Squares means with the same letter are not significantly different.

Figure 3.2 Lutein, zeaxanthin and β-carotene content in different maize-based products for sample 12



3.4.3 Effect of processing treatments on antioxidant activities (AOA) of maize

The oxygen radical absorbance capacity (ORAC) of processed maize extracts (μmol Trolox equiv./g DW) are shown in Table 3.4. For boiled maize, samples 1, 4, 5, 10, 11, and 12 maintain the same ORAC and the rest decreased by 17.6% to 32.1%. For roasted maize, except for samples 9 and 10 which showed a decrease, the ORAC values of rest of the samples did not show significant changes. For fermented maize, the ORAC value of sample 9 had a decrease of 37%, while samples 6 and 11 maintained. The rest of the samples all increased significantly ($P < 0.05$). For fermented porridge, except for samples 6 and 10 which showed a decrease, all the other samples increased significantly (P < 0.05). Unfermented porridge was similar to fermented porridge, with only sample 6 showing decreases; all of rest increased significantly (P < 0.05). These data were all collected after freeze-drying to remove any dilution factors. Different antioxidant values were also seen in an extruded tortillas study. An 87.2% to 90.7 % retention of total hydrophilic antioxidant activity was observed compared with raw kernel (Aguayo-Rojas et al., 2012). Ludwing et al. (2012) showed that the antioxidant capacity of coffee was increased after roasting, too. Chávez, Ascheri et al. (2017) also reported an increase in antioxidant capacity of sorghum after extrusion (Chávez, Ascheri, Carvalho, Godoy, & Pacheco, 2017; Ludwig et al., 2012).

The DPPH radical scavenging capacity of processed maize extracts (μmol Trolox equiv./g DW) are shown in Table 3.5. There were significant decreases in DPPH radical scavenging capacity from 33.6% to 72.3%, except for boiling treatment. For boiled maize, samples were boiled as kernel forms, and the outer layers of the kernel prevented the loss of antioxidant capacity. According to Song and Liu (2013), a significant drop of 58.9% to 74.5% of DPPH radical scavenging capacity of sweet corn kernel was observed in various cooking methods, including boiling, microwaving, and frying.

The results using oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging capacity methods were different. The main reason is that different mechanisms were involved. In the ORAC assay, the sample to be tested was mixed with a fluorescent compound, and free radicals were generated at a known rate (520 nm, in this case). The fluorescent compound was damaged when free radicals were generated. Antioxidants could mop up the free radicals generated, thus preventing the loss of the fluorescent compound (Moniruzzaman, Khalil, Sulaiman, & Gan, 2012). The higher the antioxidants in the sample, the more fluorescence would remain in the mixture. A fluorescent emission at 520 nm was read after certain time, and the difference with initial reading was used to calculate the antioxidant capacity of the food. This method is mainly based on a competitive probe reaction (Amorati & Valgimigli, 2015). The DPPH method is an indirect method where persistent colored radicals are working as probes. This method mainly relies on the satiability of a 2,2-diphenyl-1-picrylhydrazyl free radical to react with hydrogen donors. Then, any color change is measured using a spectrophotometer to indicate antioxidant capacity in food (Amorati & Valgimigli, 2015). Normally, this method is used for phenolic antioxidants, and, in this case, the color of carotenoids may have an overlap with color probes under 515 nm.

Table 3.4 Oxygen radical absorbance capacity (ORAC) of processed maize-based products (μmol Trolox equiv./g DW)

	Raw Maize	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	246.9 ±15.0^{f}	343.3 ±14.4^{cd}	307.7 ±17.1^{e}	296.9 ±17.7^{e}	307.4 ±12.3^{h}	412.1 ±17.2^{fg}
Sample 2	472.4 ±15.9^{ab}	336.2 ±15.0^{cd}	481.1 ±13.4^{b}	635.1 ±13.6^{bc}	461.9 ±16.4^{e}	565.7 ±16.5^{c}
Sample 3	437.0 ±8.0^{c}	360.1 ±19.5^{cd}	420.6 ±11.0^{bc}	922.1 ±13.6^{a}	605.2 ±14.2^{bc}	515.9 ±13.2^{d}
Sample 4	337.8 ±14.8^{d}	342.1 ±18.0^{cd}	403.9 ±15.0^{c}	469.2 ±19.7^{d}	408.3 ±12.9^{fg}	605.4 ±15.4^{b}
Sample 5	340.8 ±13.1^{d}	431.7 ±16.5^{bc}	574.4 ±12.8^{a}	495.5 ±7.0^{b}	481.8 ±14.5^{de}	335.4 ±9.7^{g}
Sample 6	506.7 ±7.5^{a}	343.8 ±10.5^{cd}	472.2 ±15.4^{b}	508.7 ±11.5^{cd}	430.9 ±12.1^{ef}	402.4 ±15.4^{fg}
Sample 7	414.6 ±14.5^{c}	259.4 ±16.9^{e}	454.5 ±16.4^{b}	696.5 ±13.5^{b}	525.9 ±19.0^{d}	487.9 ±12.2^{de}
Sample 8	293.6 ±7.2^{e}	223.2 ±17.2^{f}	423.1 ±16.6^{bc}	508.7 ±13.1^{cd}	452.5 ±14.2^{ef}	431.8 ±9.9^{f}
Sample 9	447.1 ±7.5^{ab}	316.7 ±14.0^{de}	287.0 ±14.7^{f}	287.9 ±17.0^{e}	569.1 ±14.1^{cd}	645.8 ±8.4^{ab}
Sample 10	456.8 ±10.8^{a}	500.6 ±15.4^{a}	363.4 ±7.6^{d}	479.5 ±11.4^{cd}	311.3 ±6.7^{h}	485.7 ±14.9^{de}
Sample 11	449.2 ±15.0^{c}	477.1 ±10.3^{b}	407.6 ±13.1^{c}	431.7 ±12.6^{de}	634.6 ±9.4^{b}	433.7 ±11.8^{f}
Sample 12	291.1 ±13.2^{e}	419.2 ±11.2^{bc}	378.7 ±9.9^{d}	509.8 ±12.3^{cd}	731.6 ±9.8^{a}	676.0 ±14.2^{a}

Table 3.5 DPPH radical scavenging capacity of processed maize-based products (μmol Trolox equiv./g DW)

	Raw Maize	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	5965.2±696.5^{b}	5343.0±594.1^{a}	2982.1±440.3^{ab}	2121.3±799.2^{b}	689.4±369.22^{c}	3032.7±252.4^{a}
Sample 2	5994.2±657.0^{b}	5428.5±630.8^{a}	3941.5±352.7^{a}	5549.9±152.8^{a}	3002.1±389.2^{ab}	3186.7±487.6^{a}
Sample 3	6170.6±556.0^{ab}	5494.9±538.8^{a}	3229.1±305.1^{ab}	2182.6±182.8^{ab}	3578.6±22.4^{a}	3375.7±521.2^{a}
Sample 4	5973.5±611.1^{b}	6007.3±954.2^{a}	3317.9±987.6^{ab}	2870.8±735.7^{ab}	1921.9±219.5^{abc}	3029.3±217.7^{a}
Sample 5	6384.7±314.2^{ab}	5172.1±444.9^{a}	2564.5±302.7^{b}	2991.3±633.3^{ab}	2808.3±492.0^{abc}	3253.2±610.5^{a}
Sample 6	6186.1±480.8^{ab}	6000.7±754.4^{a}	2951.2±558.6^{ab}	2128.8±968.4^{ab}	1687.6±856.7^{abc}	2900.2±197.3^{a}
Sample 7	5877.9±92.5^{b}	6077.8±862.7^{a}	3462.6±228.5^{ab}	3988.5±945.2^{ab}	3918.6±862.0^{a}	2982.8±342.1^{a}
Sample 8	6341.5±431.5^{ab}	5867.2±576.6^{a}	3420.1±305.8^{ab}	2777.9±670.0^{ab}	2629.4±767.5^{abc}	3217.5±709.3^{a}
Sample 9	6512.4±390.8^{ab}	5895.4±794.9^{a}	2885.8±518.8^{ab}	1079.7±155.4^{b}	2116.2±792.6^{abc}	2754.2±175.1^{a}
Sample 10	6238.5±338.2^{ab}	5541.5±487.6^{a}	3230.1±908.9^{ab}	2559.5±853.6^{ab}	1248.5±236.7^{bc}	2837.2±215.6^{a}
Sample 11	6363.4±342.2^{ab}	5531.5±642.4^{a}	2932.0±57.5^{ab}	1657.1±742.8^{b}	1350.2±706.3^{bc}	2598.9±99.9^{a}
Sample 12	7221.4±282.4^{a}	5640.7±565.2^{a}	2782.4±230.5^{b}	3642.3±573.4^{ab}	3763.8±772.4^{a}	3310.6±208.3^{a}

Values are means of five replicates ± SD.

Mean values having the same letters within a column are not significantly different, $P < 0.05$

Retention%=Carotenoids content after treatment/ carotenoids content before treatment

4. DETERMINATION OF CAROTENOIDS AND ANTIOXIDANT CAPACITY IN MAIZE PRODUCTS AS AFFECTED BY COOKING METHODS AND DIGESTION

4.1 Abstract

Maize has been recognized as a source of provitamin A carotenoids to improve health and prevent disease. Lutein and zeaxanthin in maize help people maintain normal vision, lower the oxidative stress, and prevent certain diseases. In this study, changes of carotenoids content, oxygen radical absorbance capacity (ORAC), and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity were determined for five maize-based products (boiled maize, roasted maize, fermented maize, fermented porridge, and unfermented porridge). These parameters were also evaluated after a three-stage in-vitro digestion of the five treatments for their digestive stability, including whole digesta and liquid digesta. Without any cooking treatments, carotenoids recovery for raw maize ranged from 73.0% to 100.5% after digestion. After cooking treatments, carotenoid recovery showed varying levels of increase, except for fermented maize, which remained low. The average carotenoids in whole digesta recovery following boiled maize, roasted maize, fermented porridge, and unfermented porridge were 108.5%, 116.9%, 101.9%, and 110.7%, respectively. In conclusion, cooking treatments can prevent the breakdown of carotenoids during digestion.

4.2 Introduction

Maize is used as one of the main staple foods in the world. Compared with other staple crops, including rice, wheat, and oats, maize has the greatest antioxidant activity (Adom & Liu, 2002). Maize also contains various types of proteins, which account for 8% to 12% of maize kernels. The digestibility of these proteins is considered to be approximately 80% to 90%. Besides the macronutrients, maize is a good source of vitamins and minerals, such as vitamins B1 and B2, zinc, and iron. Maize is also a good source of phytochemicals, including carotenoids, which have been shown to impart health benefits on consumers. Carotenoids play an important role as bioactive compounds, with lutein and zeaxanthin making up almost 70% of the total carotenoids in maize. Both α-carotene and β-carotene, as well as β-cryptoxanthin, are present in small amounts in the maize kernel, considerably less than lutein and zeaxanthin. Maize has various kinds colors ranging from white, yellow, orange, and purple, some of which are illustrative of the type of constitutive bioactive compounds. For example, yellow maize has a higher level of carotenoids and antioxidant activity than white maize (de la Parra et al., 2007).

To be absorbed and utilized by humans, carotenoids are packaged into lipid-soluble micelles. The micellarization efficiency of carotenoids is defined as the percentage of carotenoids subsequently transferred from the crude digesta to the filtered aqueous fraction during digestion. Micellarization efficiency is commonly used as a measure of relative bioaccessibility, which correlates to carotenoid bioavailability in-vivo (Failla et al., 2008). Bioavailability is defined as the fraction of carotenoids that can be absorbed and circulated by tissue from a food source. Bioaccessibility is defined as the proportion of carotenoids in mixed micelles from a food source (Kean et al., 2008).

Cooking alters the composition of the food matrix. Thus, the bioaccessibility and bioavailability of bioactive compounds are also altered. To determine which food products could serve as better carotenoid sources, the objective of this experiment was to analyze the bioaccessibility of carotenoids in five maize-based food products (boiled maize, roasted maize, fermented maize, and fermented and unfermented porridge) and their antioxidant capacity.

4.3 Materials and Methods

4.3.1 Chemical and standards

HPLC grade hexane, methyl-butyl ether (MtBE) 1-butanol, and methanol were acquired from Fisher Scientific (Whitby, ON, Canada). Carotenoid standards, lutein (≥97.0%), zeaxanthin (≥97.0%) β-Cryptoxanthin (≥97%), α-Carotene (≥95%), and β-Carotene (≥95%); Porcine pancreatic α-amylase (10 Units/mg), Porcine pepsin (25

Units/mg), Porcine pancreatin (203 USP); 2,2′azobis (2-amidinopropane) dihydrochloride (AAPH) and 2,2,-diphenyl-1-picrylhydrazyl (DPPH) (95%), and butylated hydroxytoluene (BHT) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein and Trolox (6-hydroxy-2,5,7,8-tramethylchroman-2-carboxylic acid) were acquired from Fisher Acros Organics (Morris Plains, NJ, USA). Deionized water (Mill-Q) was used in a HPLC analysis.

4.3.2 Three stages simulated human digestion system

Carotenoid bioaccessibility was analyzed using a three-stage simulated digestion, which resembled the oral, gastric, and small intestinal phase. The in-vitro digestion was performed according to Garret et al. (1999), with some modifications. For the oral phase, 1g sample was weighed into a digestion tube, to which was added 190µL water, 10µl 0.3M calcium chloride, and 0.8 mL 75 Units/mL α-amylase (prepared by adding 120 mg in 16ml simulated saliva fluid). The mixture was then homogenized with a rotational shaker and incubated at 37°C for 2 minutes. For gastric digestion, the oral phase digesta was acidified (pH 2) with 1M HCl and 1.6 ml of porcine pepsin (4000 Units/mL pepsin by adding 600mg pepsin into 40ml simulated gastric fluid). The digestion tube was incubated at 37°C, shaking at 95 rpm for 2 hours. Then, the pH of the digested meal was raised to 7.0±0.1 with 1M sodium bicarbonate, followed by the addition of a mixture of bile extract and pancreatin (9 mL containing 2 mg/mL pancreatin and 12 mg/mL bile extract in 100 mmol/L sodium bicarbonate solution).

The digestion tube was incubated at 37°C, shaking at 95 rpm for 2 hours, to complete the intestinal phase of the in-vitro digestion process. A final 10 mL aliquots of digested meal were cooled down and separate into two parts (whole digesta and liquid digesta). The whole digesta part was freeze-dried and kept under -40°C. The rest was filtered to pass through 0.45µM membrane and kept in amber tube under -40°C before extraction of carotenoids.

4.3.3 Carotenoids extraction from the whole digesta

Carotenoids extraction from whole digesta was carried out using the method described in section 3.3.5

4.3.4 Carotenoids extraction from aqueous digesta

Frozen aqueous digesta was thawed. Carotenoids were extracted according to Ferruzzi et al. (2007). Samples were incubated at 37°C for 30 mins in an incubator after adding 20µL of protease (100mg/10mL PBS). Samples were then vortexed for 45s with 500µl of sodium dodecyl sulfate (SDS)-ethanol-BHT (1.0 ml of a 20% SDS-water solution dissolved in 19.0 mL of ethanol). Each sample was then extracted three times using 500µl of petroleum ether: acetone (3:1) containing 0.1% BHT (w/v). Between each extraction, samples were centrifuged for 1 min at 10000xg to facilitate phase separation. Then, pooled extracts were dried under nitrogen and dissolved in 400µl of methyl tert-butyl ether (MTBE): methanol (50:50) for HPLC analysis.

4.3.5 Analysis of carotenoids content by using HPLC

HPLC analysis was carried out using the method described in section 3.3.6

4.3.6 Oxygen radical absorbance capacity assay (ORAC)

ORAC analysis was carried out using the method described in section 3.3.7.

4.3.7 DPPH radical scavenging capacity activity assay

DPPH analysis was carried out using the method described in section 3.3.8.

4.3.8 Statistical analysis

Results were reported as means ± standard deviation (SD) of triplicate determination. Analysis of variance (ANOVA) for main factors (maize from different location, processing method) was determined by using a GLM procedure with SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Variance was considered significant when $p < 0.05$, using least significant differences (LSD). Also, a Pearson correlation test was used to evaluate the correlation among variables at significant levels of $p < 0.05$.

4.4 Results and Discussion

4.4.1 In-vitro carotenoids bioaccessibility of different maize-based products

To determine carotenoid contents from processed maize-based products available for intestinal micellarization and absorption, representative samples of each processed maize-based product were subjected to three-stage in-vitro digestion to determine their digestive stability. The amount of carotenoids recovered in the digesta from the small intestine following digestion, compared to the amount of carotenoids in the original food, represents digestive recovery or stability (reported as a percentage) (Kean, Bordenave, Ejeta, Hamaker, & Ferruzzi, 2011).

According to Borel et al. (1996), the solubility of zeaxanthin in pure bulk triglycerides is 0.022 to 0.088 wt%, while β-carotene has a higher solubility of 0.112 to 0.141 wt%. In this chapter, the highest carotenoid content determined in the aqueous condition was 109.0μg/g, which is much lower than the carotenoid solubility (Borel et al., 1996).

Carotenoids compounds in the various extracts were separated by HPLC and were identified and quantified through comparison with appropriate standards. Results are shown in Tables 4.1a to 4.1f. To determine the effects of digestion on the carotenoid profile and bioaccessibility of processed maize-based food products, raw maize samples were also digested, and the carotenoids were analyzed. Table 4.1 shows the lutein, zeaxanthin, and β–carotene content before digestion, as well as in the whole digesta and aqueous digesta after digestion for raw and processed maize-based products (boiled maize, roasted maize, fermented maize, and fermented porridge and unfermented porridge). In this study, five carotenoids were analyzed, but only three are presented (lutein, zeaxanthin, and β–carotene), due to the very low relative amounts of α-carotene and β–cryptoxanthin.

For unprocessed maize samples (raw), after digestion, the levels of lutein, zeaxanthin, and β–cryptoxanthin were reduced by 17.5%, 24.6%, and 17.7% on average for whole digesta (Table 4.1a). The changes in carotenoid content following digestion showed no significant difference among three genetic groups. For boiled samples, lutein and zeaxanthin increased by 2.1% and 6.4%, respectively, and β–carotene increased by 85.5% on average after digestion for the whole digesta (Table 4.1b). In the roasted samples, the levels of lutein, zeaxanthin, and β-carotene in whole digesta increased. After the digestion of fermented maize samples, lutein and zeaxanthin were reduced, reaching levels below that of the raw maize samples. Only β-carotene showed an increase of 56.0% — for the carotenoids content of whole digesta of fermented porridge samples. The retention rates of lutein and zeaxanthin were 102.9% and 90.9%, respectively. β-carotene had the highest retention rate of 172.5%. In contrast, the retention rate of lutein, zeaxanthin, and β-carotene in whole digesta of unfermented porridge were 113.4%, 108.0%, and 146.1%, respectively.

Thermal processing helps to improve carotenoids bioaccessibility and bioavailability. During processing, the food matrix softens, cellular integrity is lost, and the protein-carotenoid complexes break. These processes could increase carotenoids extractability during digestion (Yahia et al., 2017), which explains the current findings that some of the processed foods showed a higher carotenoids content compared to unprocessed foods.

The content of soluble carotenoids in raw and processed maize are shown in Tables 4.1a to 4.1f. Unlike carotenoids in the whole digesta, carotenoids in the liquid phase had a similar retention rate of 32% to 35.9% for raw, boiled, roasted, and fermented maize. Retention rates of soluble carotenoids in fermented (26.1%) and unfermented (19.4%) porridge were lower than the retention rates in other treatments.

Digestive recovery was determined by comparing the carotenoid content in the final digested product to the original maize-based products. Digestive recoveries for total carotenoids ranged from 60.2% to 100%. Compared to different treatments, the recovery of carotenoids following boiled maize, roasted maize, and fermented maize ranged from 94.8% to 100%, and the carotenoids recovery of fermented and unfermented porridge ranged from 60.2% to 78.2%. Kean et al. (2008) qualitatively and quantitatively determined the carotenoid profiles in bread, extruded puff, and porridge made with maize, and the obtained digestive recoveries ranged from 90% to 120% among these products. However, since they used different extraction methods between digestive products and original food products, the real recovery could be lower than their data suggests, which makes the current results more comparable (Kean, Hamaker, & Ferruzzi, 2008). Tukey-Kramer Grouping was used for sorting the treatments based on carotenoids in digesta. Using lutein content in whole digesta after digestion as an example, lutein level was not significantly different across fermented maize, roasted maize, boiled maize, and fermented porridge (refer to Table 4.1) The carotenoids content for fermented maize was significantly higher than that of raw or unfermented porridge.

The efficiency of micellarization for carotenoids from digested maize-based products varied among treatments. In general, carotenoids micellarization ranged from 29.5% to 46% for boiling, 28.3% to 37.6% for roasted maize, 26.4% to 30.7% for fermented maize, 21.48% to 26.48% for fermented porridge, and 21.6% to 29.6 for unfermented porridge. These results agree with Ferruzzi et al. (2006), in which the efficiency of micellarization of the *trans* and *cis* isomer of β-carotene was quantified using a maize-based meal. Based on their observation, the micellarization of cis-β-carotene was 12% to 24% of digesta. Similar results can be found in a report by Kean et al. (2008), where different processing methods were applied and micellarization efficiency of carotenoids ranged from 59% to 67% for bread and 45% to 50% for porridge. The difference between current results and other literature is likely due to differences in the types of maize samples, processing techniques, or treatments, as well as the differing extraction methods.

In food matrices, carotenoids are usually present as a complex. For example, carotenes are found complexed with protein in chromoplasts (Garrett, Failla, & Sarama, 2000). Carotenoid bioaccessibility can be affected by the structure of the food matrix. Some food processing, such as pasteurization, microwaving, or thermal cooking, can soften cell walls, break protein-carotenoid complex, and release carotenoids, which makes digestive enzymes work more efficiently (Cilla, 2018). After releasing from complexes, carotenoids are incorporated with lipid droplets, after which they enter micelles and are absorbed by the intestinal epithelium (Etcheverry, Grusak, & Fleige, 2012). According to Cervantes-Paz et al. (2016), only carotenoids released from the food matrix can be available for absorption and utilization. Forming a complex with emulsified gastrointestinal contents can help carotenoids reach enterocytes and impart health benefits (Cervantes-Paz, Victoria-Campos, & de Jesús Ornelas-Paz, 2016). After uptake by intestinal epithelium, some esterified xanthophyll can be transported through gastrointestinal aqueous by a lipid transporter SR-BI. Xanthophylls (*i.e.* lutein, zeaxanthin) and β-carotene can be absorbed by human retinal pigment epithelial (ARPE-19) cells, then transported to the human retina (During, Doraiswamy, & Harrison, 2008). Moreover, lutein and zeaxanthin can accumulate in the retina and lens of the eye, which contributes to filtering the blue light and thus preventing damage of the eyes. Some researchers also reported that carotenoids have functions of inhibiting the growth of human cancer cells located in organs such as the colon, liver, breast, and skin (Ford, Elsen, Zuniga, Lindshield, & Erdman, 2011; Mucci et al., 2001).

According to the National Institute of Health (NIH), the recommended dietary allowance (RDA) of vitamin A is 900 µg retinol activity equivalent (RAE) per day for men, and 700µg RAE per day for women. To meet men’s recommended dietary allowance, boiled maize is most efficient, containing the most carotenoids among five maize-based products (Table 4.1d), and, therefore, a minimal intake of 6.57g per day would meet the recommendation. Unfermented porridge contained the least carotenoids among five maize-based products (Table 4.1f). As such, the minimal intake of 11.64g per day for unfermented porridge is recommended. To meet women’s recommended dietary allowance, boiled maize is most effective, containing the most carotenoids among five maize-based products (Table 4.1d), and, therefore, a minimal intake of 5.11g per day would meet the recommendation. Unfermented porridge contained the least carotenoids among five maize-based products (Table 4.1f). As such, a minimal intake of 9.05g per day for unfermented porridge is recommended.

Table 4.1a Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Raw Maize)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	70.0 ± 0.9^{e}	61.7 ± 2.3^{g}	22.6 ± 2.5^{d}	46.2 ± 2.8^{cd}	19.4 ± 1.1^{e}	6.6 ± 0.4^{e}	3.1 ± 0.0^{b}	8.8 ± 0.8^{e}	2.1 ± 0.0^{d}
Sample 2	89.7 ± 9.4^{e}	82.7 ± 15.0^{fg}	37.4 ± 8.3^{cd}	51.9 ± 3.5^{cd}	28.9 ± 3.6^{de}	13.3 ± 2.3^{e}	10.5 ± 4.9^{ab}	10.3 ± 0.6^{de}	2.2 ± 0.5^{d}
Sample 3	129.3 ± 8.6^{e}	110.5 ± 6.7^{ef}	43.4 ± 3.6^{cd}	36.6 ± 3.1^{d}	32.5 ± 0.9^{d}	12.9 ± 1.1^{e}	12.0 ± 2.0^{ab}	16.4 ± 1.6^{cd}	5.4 ± 1.1^{cd}
Sample 4	142.7 ± 7.5^{de}	111.3 ± 14.3^{ef}	49.4 ± 8.4^{bcd}	37.6 ± 3.04^{d}	30.6 ± 3.0^{de}	14.3 ± 2.3^{de}	10.0 ± 0.7^{ab}	13.1 ± 0.7^{de}	4.1 ± 0.8^{cd}
Sample 5	134.9 ± 23.4^{de}	82.2 ± 9.6^{fg}	41.9 ± 2.4^{cd}	48.4 ± 19.1^{cd}	30.2 ± 1.6^{de}	14.2 ± 0.7^{de}	11.9 ± 7.3^{ab}	12.7 ± 1.7^{de}	2.1 ± 0.8^{d}
Sample 6	174.1 ± 19.6^{cd}	129.4 ± 14.8^{de}	58.5 ± 7.1^{bc}	58.6 ± 19.43^{cd}	35.6 ± 0.9^{d}	15.7 ± 1.6^{de}	19.8 ± 6.0^{a}	16.9 ± 0.6^{cd}	4.6 ± 1.2^{cd}
Sample 7	196.0 ± 27.7^{bc}	146.5 ± 20.6^{cd}	58.7 ± 3.2^{bc}	80.9 ± 16.5^{bc}	73.2 ± 7.6^{bc}	28.9 ± 1.3^{bc}	17.6 ± 0.0^{ab}	21.0 ± 4.5^{bc}	1.7 ± 0.7^{d}
Sample 8	206.8 ± 6.2^{abc}	178.3 ± 8.3^{bc}	77.5 ± 0.5^{ab}	75.4 ± 3.0^{cd}	62.2 ± 3.1^{c}	26.6 ± 0.0^{cd}	16.0 ± 0.2^{ab}	21.6 ± 1.8^{bc}	4.6 ± 0.4^{bcd}
Sample 9	241.0 ± 4.6^{ab}	222.8 ± 1.8^{a}	77.6 ± 15.9^{ab}	95.7 ± 0.6^{ab}	85.3 ± 1.3^{a}	29.4 ± 5.9^{bc}	17.1 ± 1.1^{ab}	27.1 ± 0.7^{ab}	2.8 ± 1.9^{d}
Sample 10	226.5 ± 10.9^{ab}	196.7 ± 8.4^{ab}	102.5 ± 15.6^{a}	104.6 ± 7.0^{a}	88.1 ± 4.4^{a}	45.0 ± 7.0^{a}	19.8 ± 1.6^{a}	26.8 ± 2.3^{ab}	10.2 ± 1.7^{a}
Sample 11	244.4 ± 11.5^{a}	218.1 ± 11.6^{a}	109.0 ± 12.1^{a}	97.1 ± 4.7^{ab}	85.3 ± 7.0^{a}	41.9 ± 4.6^{a}	17.3 ± 1.9^{ab}	28.5 ± 1.8^{a}	10.1 ± 2.4^{ab}
Sample 12	254.5 ± 15.3^{a}	206.8 ± 7.3^{ab}	97.9 ± 15.1^{a}	103.7 ± 7.1^{a}	82.0 ± 4.6^{ab}	40.0 ± 6.4^{ab}	19.4 ± 3.4^{a}	25.5 ± 4.9^{ab}	9.3 ± 3.1^{abc}

Table 4.1b Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Boiled Maize)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	86.0±5.7^{g}	78.1±14.6^{gh}	37.5±3.5^{ab}	16.6±1.0^{e}	15.0±2.4^{g}	7.3±0.6^{d}	8.1±1.6^{b}	8.5±0.4^{f}	2.2±0.8a
Sample 2	38.8±10.4^{f}	52.6±1.4^{h}	20.6±2.1^{b}	19.6±3.6^{de}	28.2±0.6^{f}	11.2±0.6^{cd}	5.0±1.4 ^{ab}	11.5±0.4^{ef}	3.3±0.7a
Sample 3	103.3±26.5^{f}	113.5±0.1^{ef}	49.2±5.7^{ab}	24.9±5.6^{de}	30.3±0.3^{f}	12.6±1.5^{cd}	6.2±1.5^{ab}	14.6±0.5^{de}	4.7±0.6^{a}
Sample 4	111.3±4.8^{ef}	96.0±14.6^{de}	43.9±4.2^{ab}	31.0±1.6^{cd}	26.8±2.7^{f}	13.5±0.6^{cd}	10.0±2.4 ^{ab}	12.7±0.5^{def}	4.7±0.9^{a}
Sample 5	139.3±2.2^{cd}	116.9±9.8^{ef}	37.4±3.8^{ab}	55.9±1.5^{b}	49.7±4.3^{cd}	15.4±1.6^{bc}	11.9±2.0 ^{ab}	20.3±2.4^{ab}	2.1±0.3^{a}
Sample 6	147.7±8.0^{cd}	140.9±11.2^{de}	43.6±2.5^{ab}	40.7±2.4^{c}	43.6±4.9^{e}	13.6±0.8^{cd}	11.3±2.8 ^{b}	15.9±0.2^{cd}	2.9±0.8^{a}
Sample 7	165.0±3.0^{bcd}	188.8±4.5^{ab}	41.0±3.2^{ab}	71.5±1.2^{a}	85.8±3.9^{a}	26.0±1.3^{a}	11.9±0.3 ^{ab}	25.1±1.3^{a}	3.3±1.9^{a}
Sample 8	141.1±19.8^{de}	162.4±0.1^{bcd}	47.5±10.1^{ab}	42.5±6.4^{c}	52.6±0.6^{cde}	14.5±3.0^{bcd}	9.7±4.1 ^{ab}	20.0±1.1^{bc}	1.4±0.1^{a}
Sample 9	180.6±10.1^{abc}	151.6±0.9^{cd}	53.9±5.7^{ab}	70.9±4.9^{a}	59.8±0.6^{cd}	21.7±2.2^{ab}	12.6±2.3^{a}	22.3±1.1^{ab}	4.8±1.9^{a}
Sample 10	154.1±9.4^{bcd}	169.2±8.7^{bc}	45.2±4.8^{ab}	72.8±4.9^{a}	72.7±1.4^{b}	22.0±1.3^{ab}	10.9±1.9 ^{ab}	24.0±1.6^{ab}	3.4±0.5^{a}
Sample 11	188.4±11.1^{ab}	180.1±11.2^{ab}	50.1±10.5^{ab}	61.9±6.4^{ab}	61.2±7.5^{c}	18.5±4.0^{ab}	12.7±1.3 ^{ab}	24.8±3.3^{a}	5.0±0.9^{a}
Sample 12	189.9±6.2^{a}	198.1±7.6^{a}	64.6±17.6^{a}	72.9±4.2^{a}	80.6±2.8^{ab}	25.4±6.8^{a}	11.6±1.5^{a}	24.0±0.3^{ab}	4.4±2.5^{a}

Table 4.1c Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Roasted Maize)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	54.9 ±17.9^{ef}	73.9 ±2.1^{cd}	18.1 ±5.6^{b}	15.2 ±5.5^{e}	19.9 ±0.3^{f}	5.4 ±1.6^{b}	4.5 ±1.3 ^{d}	6.7 ±0.7^{f}	1.3 ±0.1^{a}
Sample 2	33.9 ±8.1^{f}	62.7 ±3.9^{d}	13.7 ±0.3^{b}	21.4 ±2.3^{de}	40.1 ±3.0^{bcd}	8.2 ±0.1^{ab}	6.9 ±0.9 ^{cd}	8.8 ±0.2^{ef}	1.3 ±0.0^{a}
Sample 3	55.4 ±9.5^{ef}	55.7 ±5.4^{d}	15.4 ±1.1^{b}	32.4 ±3.9^{de}	30.0 ±9.7^{def}	9.4 ±0.4^{ab}	14.2 ±2.7 ^{a}	13.7 ±2.2^{bcd}	2.5 ±0.7^{a}
Sample 4	73.1 ±6.8^{de}	87.9 ±8.9^{cd}	17.0 ±4.9^{b}	20.1 ±1.5^{e}	23.9 ±1.9^{ef}	5.3 ±1.3^{b}	5.6 ±0.6 ^{d}	8.2 ±1.6^{ef}	2.5 ±1.2^{a}
Sample 5	109.1 ±7.2^{bc}	114.8 ±20.5^{abc}	45.4 ±10.0^{ab}	33.7 ±2.7^{b}	37.8 ±9.2^{cde}	15.4 ±3.1^{ab}	10.9 ±2.0 ^{abc}	12.5 ±0.0^{cde}	3.2 ±0.6^{a}
Sample 6	95.8 ±13.3^{cd}	77.6 ±3.4^{cd}	26.8 ±0.4^{ab}	27.2 ±3.7^{ced}	23.6 ±1.7^{ef}	8.7 ±0.6^{ab}	6.4 ±0.8 ^{cd}	9.4 ±0.7^{def}	1.6 ±0.7^{a}
Sample 7	75.3 ±3.9^{de}	84.9 ±20.8^{cd}	17.8 ±5.0^{b}	39.4 ±2.4^{bc}	47.8 ±9.2^{bcd}	9.9 ±2.1^{ab}	9.1 ±2.0 ^{bcd}	12.8 ±0.5^{bcd}	1.2 ±0.1^{a}
Sample 8	78.8 ±10.6^{cde}	92.5 ±5.8^{bcd}	14.2 ±7.7^{b}	37.5 ±5.6 ^{bc}	40.5 ±6.1^{dce}	7.6 ±3.2^{ab}	7.6 ±2.0 ^{bcd}	10.7 ±0.9^{def}	-
Sample 9	152.3 ±12.9^{a}	136.0 ±16.7^{ab}	54.2 ±7.4^{a}	54.0 ±3.8^{a}	48.9 ±4.0^{bc}	21.7 ±3.2^{a}	11.0 ±2.0 ^{abc}	14.4 ±3.3^{bcd}	3.1 ±2.4^{a}
Sample 10	132.2 ±13.2^{ab}	143.6 ±21.4^{a}	44.5 ±11.3^{ab}	56.5 ±6.1^{a}	63.7 ±8.9^{ab}	18.3 ±4.5^{ab}	8.6 ±1.6 ^{bcd}	15.9 ±3.0^{ab}	4.0 ±2.8^{a}
Sample 11	147.7 ±3.2^{a}	155.3 ±6.4^{a}	53.6 ±3.7^{a}	55.7 ±0.9^{a}	68.8 ±2.1^{a}	22.8 ±9.8^{a}	10.7 ±1.6 ^{abc}	17.5 ±0.4^{ab}	3.5 ±3.8^{a}
Sample 12	108.43 ±13.5^{bc}	121.5 ±9.5^{ab}	45.5 ±2.6^{ab}	55.17 ±6.54^{a}	61.8 ±5.5^{ab}	21.5 ±13.5^{a}	12.13 ±1.57 ^{ab}	18.8 ±1.1^{a}	8.2 ±2.3^{a}

Table 4.1d Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Fermented Maize)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	49.7 ± 0.7^{g}	25.3 ± 1.1^{e}	47.7 ± 3.8^{a}	13.4 ± 1.4^{gh}	7.8 ± 0.0^{c}	16.9 ± 1.2^{ab}	3.2 ± 0.2^{f}	-	1.3 ± 0.1^{b}
Sample 2	24.9 ± 2.4^{h}	16.8 ± 6.5^{e}	16.9 ± 6.2^{bcd}	11.4 ± 0.7^{h}	7.1 ± 0.0^{c}	8.9 ± 3.1^{bc}	3.1 ± 0.5^{f}	-	-
Sample 3	32.8 ± 4.9^{h}	19.4 ± 0.3^{e}	6.7 ± 1.4^{d}	17.3 ± 1.7^{g}	8.4 ± 0.0^{c}	20.5 ± 0.4^{b}	3.4 ± 0.9^{f}	-	2.2 ± 0.4^{ab}
Sample 4	112.9 ± 3.8^{e}	87.3 ± 6.5^{cd}	37.5 ± 5.1^{abc}	36.5 ± 1.4^{f}	31.5 ± 12.7^{abc}	16.7 ± 2.6^{ab}	6.7 ± 3.1^{cde}	11.0 ± 7.1^{abc}	2.6 ± 1.8^{ab}
Sample 5	138.9 ± 7.7^{d}	103.0 ± 4.6^{bc}	7.7 ± 3.7^{d}	54.6 ± 2.5^{d}	37.3 ± 2.2^{abc}	3.7 ± 1.5^{c}	8.7 ± 1.6^{cde}		1.9 ± 0.1^{ab}
Sample 6	168.0 ± 5.4^{b}	161.0 ± 10.9^{a}	22.4 ± 9.3^{abd}	52.2 ± 1.8^{de}	50.1 ± 5.1^{ab}	7.8 ± 2.6^{bc}	10.9 ± 0.1^{abc}	16.5 ± 1.2^{a}	3.1 ± 2.4^{ab}
Sample 7	79.6 ± 4.7^{f}	44.5 ± 7.2^{de}	7.2 ± 1.0^{d}	39.3 ± 1.8^{f}	21.1 ± 1.3^{c}	3.9 ± 0.3^{c}	4.8 ± 0.9^{ef}	3.1 ± 0.4^{c}	1.2 ± 0.1^{b}
Sample 8	153.5 ± 4.3^{c}	84.7 ± 6.7^{cd}	10.2 ± 2.0^{d}	48.7 ± 2.9^{e}	26.4 ± 1.8^{bc}	3.7 ± 0.5^{c}	8.8 ± 0.8^{bcd}	7.8 ± 2.9^{bc}	2.1 ± 0.9^{ab}
Sample 9	168.8 ± 7.2^{b}	129.4 ± 2.9^{abc}	28.8 ± 7.6^{abc}	67.5 ± 2.5^{c}	52.2 ± 0.7^{a}	12.3 ± 2.7^{abc}	7.3 ± 0.5^{bcd}	9.4 ± 1.8^{abc}	1.4 ± 0.2^{ab}
Sample 10	173.1 ± 2.2^{b}	-	19.4 ± 1.7^{bcd}	78.2 ± 1.9^{b}	-	9.3 ± 0.5^{bc}	11.6 ± 0.3^{ab}	-	3.2 ± 0.1^{ab}
Sample 11	209.8 ± 4.9^{a}	144.0 ± 4.9^{ab}	41.1 ± 2.5^{ab}	86.9 ± 1.0^{a}	56.2 ± 15.7^{a}	16.4 ± 9.3^{ab}	12.8 ± 1.4^{a}	11.9 ± 3.5^{ab}	6.1 ± 2.2^{a}
Sample 12	201.6 ± 2.4^{a}	87.4 ± 12.4^{cd}	12.3 ± 4.9^{cd}	65.3 ± 0.4^{c}	26.1 ± 3.5^{bc}	4.6 ± 1.6^{c}	10.1 ± 1.1^{abc}	5.6 ± 0.8^{bc}	1.5 ± 0.4^{b}

Table 4.1e Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Fermented Porridge)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	51.1 ±4.2^{g}	142.4 ±2.3^{cd}	7.3 ±2.2^{c}	36.0 ±1.0^{e}	61.4 ±0.6^{c}	2.6 ±0.3^{e}	2.0 ±0.0^{d}	6.5 ±1.5^{b}	1.1 ±0.6^{b}
Sample 2	22.3 ±0.1^{f}	148.5 ±7.2^{bcd}	7.9 ±0.8^{c}	12.9 ±0.4^{cd}	65.6 ±2.7^{bc}	4.4 ±0.3^{de}	2.6 ±0.0^{cd}	2.5 ±0.9^{c}	1.1 ±0.1^{ab}
Sample 3	61.1 ±16.8^{f}	20.3 ±1.8^{f}	15.4 ±4.4^{c}	37.6 ±10.3^{e}	13.8 ±2.4^{e}	10.8 ±2.9^{cde}	2.1 ±1.7^{d}	3.1 ±0.0^{d}	1.7 ±0.2^{ab}
Sample 4	105.9 ±3.8^{e}	164.3 ±3.8^{abc}	20.2 ±0.3^{c}	30.8 ±1.0^{d}	62.9 ±2.0^{c}	7.2 ±0.1^{de}	4.5 ±0.6^{cd}	17.7 ±0.6^{b}	2.1 ±0.7^{ab}
Sample 5	113.9 ±6.7^{de}	31.3 ±1.2^{ef}	55.8 ±2.0^{ab}	44.0 ±17.3^{cd}	9.9 ±1.6^{e}	21.9 ±0.1^{ab}	5.9 ±2.0^{c}	4.5 ±1.1^{d}	-
Sample 6	155.4 ±4.3^{cd}	114.2 ±27.9^{d}	46.6 ±10.7^{b}	47.3 ±0.8^{cd}	33.4 ±4.8^{d}	15.0 ±3.1^{bc}	9.2 ±0.2^{b}	9.9 ±0.2^{c}	4.8 ±1.5^{a}
Sample 7	156.8 ±5.0^{cd}	61.0 ±11.6^{e}	15.6 ±2.3^{c}	60.0 ±1.2^{b}	40.3 ±2.3^{d}	8.5 ±0.6^{cd}	9.3 ±0.0^{b}	4.6 ±0.1^{d}	1.4 ±0.3^{ab}
Sample 8	153.2 ±0.0^{cd}	112.4 ±8.8^{d}	63.6 ±5.6^{ab}	58.8 ±0.0^{bc}	16.4 ±7.8^{e}	26.2 ±2.0^{a}	12.4 ±0.0^{ab}	-	2.2 ±0.6^{ab}
Sample 9	183.7 ±2.0^{bc}	185.6 ±18.1^{ab}	47.3 ±2.0^{b}	76.7 ±1.3^{ab}	85.4 ±3.8^{a}	20.9 ±.7^{ab}	9.3 ±0.3^{b}	16.0 ±0.9^{b}	1.4 ±0.1^{ab}
Sample 10	166.4 ±1.2^{c}	158.6 ±1.1^{abc}	52.4 ±4.5^{ab}	74.7 ±0.8^{ab}	76.0 ±0.1^{abc}	25.3 ±2.2^{a}	9.2 ±0.6^{b}	16.9 ±0.6^{b}	1.5 ±0.1^{ab}
Sample 11	219.5 ±5.4^{ab}	189.4 ±12.6^{a}	55.1 ±8.7^{ab}	89.6 ±2.2^{a}	79.4 ±5.1^{ab}	23.7 ±3.9^{a}	14.3 ±0.3^{a}	20.9 ±1.0^{a}	3.5 ±1.6^{ab}
Sample 12	224.0 ±0.4^{a}	162.5 ±23.8^{abc}	71.4 ±13.5^{a}	77.0 ±0.9^{ab}	63.5 ±13.3^{c}	25.3 ±4.8^{a}	12.8 ±0.5^{a}	18.7 ±1.2^{ab}	3.1 ±1.6^{ab}

Table 4.1f Lutein, zeaxanthin and β-carotene content of food matrix before digestion, whole digesta and water soluble digesta of different maize based food products (μg/g DW) (Unfermented Porridge)

	lutein			Zeaxanthin			β-carotene		
	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta	Food matrix	Whole digesta	Water soluble digesta
Sample 1	81.1 ±1.3^{cd}	96.3 ±10.2^{bcd}	12.4 ±4.0^{cd}	23.2 ±0.5^{d}	30.5 ±5.2^{c}	4.4 ±0.8^{cd}	4.7 ±1.3^{bcd}	10.1 ±0.7^{abc}	1.4 ±0.3^{a}
Sample 2	22.7 ±5.1^{f}	56.3 ±16.7^{de}	7.0 ±2.3^{cd}	12.9 ±1.5^{e}	-	4.1 ±1.2^{cd}	3.1 ±0.0^{d}	5.2 ±0.2^{c}	-
Sample 3	71.0 ±1.7^{de}	62.5 ±10.4^{cde}	16.1 ±6.2^{cd}	33.0 ±1.0^{cd}	32.9 ±5.9^{c}	7.1 ±0.7^{bc}	7.1 ±2.3^{bcd}	8.0 ±0.9^{cb}	-
Sample 4	107.3 ±3.3^{c}	125.4 ±15.2^{bc}	19.0 ±6.3^{bc}	25.7 ±0.9^{d}	36.8 ±6.6^{c}	5.6 ±1.1^{cd}	6.3 ±1.7^{cd}	11.0 ±2.2^{abc}	1.8 ±0.1^{a}
Sample 5	104.0 ±33.1^{c}	139.3 ±7.2^{ab}	29.2 ±3.4^{ab}	38.5 ±10.5^{c}	53.3 ±0.9^{b}	11.5 ±1.3^{a}	9.2 ±2.3^{bcd}	17.3 ±0.4^{abc}	3.4 ±1.6^{a}
Sample 6	103.2 ±7.3 ^{c}	118.5 ±9.6^{bcd}	34.7 ±4.4^{a}	30.4 ±1.3 ^{d}	41.7 ±12.2^{bc}	10.9 ±1.3^{a}	6.2 ±2.1 ^{cd}	13.3 ±8.9^{abc}	1.7 ±0.2^{a}
Sample 7	61.7 ±8.7^{de}	65.9 ±3.5^{cde}	14.4 ±1.9^{cd}	40.0 ±2.5^{c}	31.3 ±1.2^{c}	9.8 ±0.7^{ab}	3.4 ±0.6^{d}	3.0 ±0.1^{c}	-
Sample 8	46.8 ±5.0^{ef}	24.6 ±5.4^{e}	13.4 ±0.4^{cd}	23.1 ±0.9	14.0 ±2.9^{d}	7.1 ±0.2^{bc}	4.0 ±1.4^{d}	3.8 ±1.5^{c}	-
Sample 9	181.6 ±2.6^{a}	205.8 ±5.4^{a}	10.8 ±1.1^{cd}	62.6 ±1.2^{b}	72.2 ±2.2^{a}	3.1 ±0.2^{d}	11.0 ±0.5^{ab}	17.3 ±1.1^{ab}	1.3 ±0.1^{a}
Sample 10	150.2 ±4.1^{b}	167.2 ±8.7^{ab}	29.4 ±8.3^{ab}	63.3 ±1.4^{b}	72.6 ±4.1^{a}	9.5 ±2.4^{ab}	10.4 ±1.4^{abc}	18.5 ±0.9^{a}	4.4 ±0.0^{a}
Sample 11	175.1 ±2.0^{ab}	168.1 ±18.2^{ab}	5.3 ±1.9^{d}	73.7 ±1.1^{a}	75.9 ±3.8^{a}	3.5 ±1.2^{d}	11.6 ±0.7^{a}	15.5 ±1.8^{abc}	-
Sample 12	102.6 ±1.8^{c}	62.1 ±12.0^{cde}	3.9 ±0.7^{d}	44.1 ±1.2^{c}	28.7 ±3.5^{cd}	2.2 ±0.4^{d}	3.5 ±0.4	4.1 ±0.5^{c}	-

Values are means of three replicates ± SD.

- Not detected.

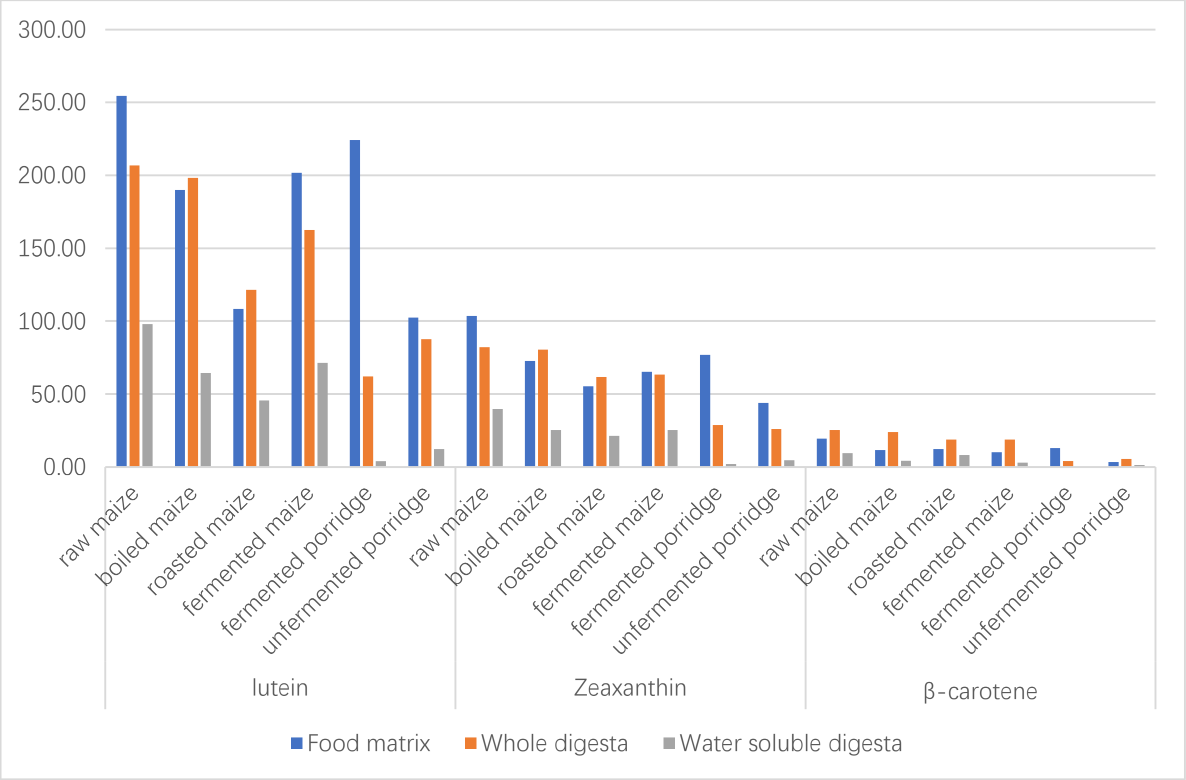
Mean values having the same letters within a column are not significantly different, $P < 0.05$

Table 4.2 Tukey-Kramer Grouping for treatments Least Squares Means (Alpha=0.05)

Treatment	**Average carotenoids content for lutein (µg/g DW)**		
Raw Maize	145.63		A
Boiled Maize	136.90	B	A
Fermented Maize	116.45	B	C
Fermented Porridge	104.95		C
Roasted Maize	100.66		C
Unfermented Porridge	77.32	D	

Least Squares means with the same letter is not significantly different.

Figure 4.1 Lutein, zeaxanthin and β-carotene content after different treatments and digestion for sample 12



4.4.2 In-vitro antioxidant potential of different maize-based products

The oxygen radical absorbance capacities (ORAC) of the whole digesta in different treated samples after digestion are shown in Table 4.3, and aqueous digesta in different treated samples after digestion is shown in Table 4.4. The antioxidant capacity of all samples exhibited significant reduction. On average, retention of ORAC values was 23.1% after boiling, 18.4% after roasting, 22.8% after fermentation, 20.1% in fermented porridge, and 19.6% in unfermented porridge. ORAC values of whole digesta after digestion had no significant differences among the five treatments. ORAC values of soluble digesta were also analyzed. On average, ORAC values in soluble digesta were 11.6% for boiled maize, 11.1% for roasted maize, 11.8% for fermented maize, 11.0% for fermented porridge, and 10.1% for unfermented porridge samples.

Tables 4.5 and 4.6 summarize the DPPH radical scavenging capacity of whole digesta and aqueous digesta of different treatments, respectively. Unlike ORAC, the DPPH of whole digesta for fermentation were higher than other treatments and had the highest retention rate of 23.6%. Boiled samples had the lowest DPPH retention rate of 8.2%. DPPH of whole digesta of raw samples were also analyzed and compared with DPPH values before digestion. The retention rate was 12.1%, which was higher than for boiled maize (8.2%) and lower than for roasted maize (16.9%), fermented maize (23.6%), fermented porridge (16.9%), and unfermented porridge (14.6%).

The antioxidant activity of carotenoids has been extensively studied. The conjugated double bonds in carotenoids structure and electron-rich systems contribute to its antioxidant activity. Based on animal studies, there are three major chemical reactions to quench singlet oxygen and peroxyl radicals. Therefore, carotenoids can help with deactivation of electronically excited sensitizer molecules and filter blue light (Van den Berg et al., 2000). Some epidemiological studies also show some carotenoids health benefits, such as reducing risk of cardiovascular diseases, functions associated with their antioxidant activity regarding action mechanism (Bub et al., 2000). Another study of rheumatoid arthritis also showed that higher antioxidants consumption using carotenoids can reduce the risk of rheumatoid arthritis (Costenbader, Kang, & Karlson, 2010).

Table 4.3 Oxygen radical absorbance capacity (ORAC) of whole digesta in different processed foods after digestion (µmol Trolox equiv./g DW)

	Raw	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	106.2 ±12.2^{a}	119.2 ±8.1^{a}	52.5 ±6.4^{b}	92.8 ±8.6^{b}	83.2 ±16.4^{cd}	112.4 ±12.1^{a}
Sample 2	107.8 ±18.1^{a}	66.3 ±8.8^{b}	81.4 ±14.2^{a}	102.3 ±22.5^{ab}	102.8 ±14.7^{bcd}	84.9 ±11.8^{bc}
Sample 3	101.2 ±20.7^{a}	63.7 ±7.2^{b}	77.4 ±11.0^{a}	102.4 ±18.3^{ab}	101.9 ±16.9^{bcd}	93.7 ±13.8^{ab}
Sample 4	111.6 ±20.5^{a}	119.5 ±6.7^{a}	67.0 ±6.6^{ab}	97.0 ±14.6^{ab}	107.0 ±15.4^{abc}	92.6 ±8.5^{ab}
Sample 5	97.1 ±15.6^{a}	72.8 ±8.9^{b}	80.1 ±13.3^{a}	106.5 ±9.7^{ab}	82.2 ±3.2^{cd}	102.8 ±10.9^{ab}
Sample 6	103.5 ±4.4^{a}	66.9 ±6.7^{b}	65.2 ±7.6^{ab}	107.3 ±21.4^{ab}	97.7 ±6.4^{bcd}	97.6 ±19.4^{ab}
Sample 7	90.9 ±9.7^{a}	81.8 ±9.1^{b}	75.1 ±12.7^{a}	104.7 ±12.2^{ab}	100.7 ±12.0^{bcd}	95.9 ±12.4^{ab}
Sample 8	93.5 ±13.0^{a}	79.1 ±9.6^{b}	73.4 ±4.6^{ab}	130.4 ±11.3^{a}	77.9 ±7.9^{d}	77.1 ±12.3^{cd}
Sample 9	106.2 ±21.0^{a}	68.4 ±8.9^{b}	82.1 ±15.4^{a}	112.1 ±13.0^{ab}	87.7 ±4.6^{cd}	89.8 ±16.6^{ab}
Sample 10	102.3 ±6.8^{a}	64.9 ±5.3^{b}	79.6 ±10.2^{a}	118.2 ±23.4^{ab}	97.2 ±10.8^{bcd}	84.6 ±18.7^{bc}
Sample 11	93.0 ±7.9^{a}	67.2 ±12.9^{b}	74.9 ±20.0^{a}	106.6 ±25.7^{ab}	118.1 ±19.7^{ab}	110.0 ±10.3^{ab}
Sample 12	109.2 ±20.6^{a}	78.4 ±16.9^{b}	80.9 ±7.0^{ab}	115.0 ±23.1^{ab}	129.6 ±15.2^{a}	71.3 ±14.7^{d}

Table 4.4 Oxygen radical absorbance capacity (ORAC) of liquid digesta in processed foods after digestion (μmol Trolox equiv./g DW)

	Raw	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	42.7 ±11.5^{a}	43.6 ±7.1^{ab}	52.0 ±7.0^{a}	57.4 ±5.3^{ab}	39.4 ±9.9^{c}	45.6 ±11.1^{ab}
Sample 2	51.6 ±7.1^{a}	47.1 ±10.0^{a}	44.1 ±8.1^{a}	79.3 ±10.4^{a}	39.2 ±5.8^{c}	56.1 ±8.1^{ab}
Sample 3	49.3 ±10.0^{a}	42.9 ±8.7^{ab}	45.5 ±10.7^{a}	56.8 ±7.2^{ab}	41.6 ±6.8^{c}	54.6 ±19.6^{ab}
Sample 4	42.9 ±5.0^{a}	37.0 ±0.5^{ab}	51.3 ±12.2^{a}	56.5 ±7.2^{ab}	40.8 ±11.4^{c}	48.7 ±17.3^{ab}
Sample 5	49.7 ±6.1^{a}	37.2 ±9.2^{ab}	44.9 ±12.1^{a}	39.5 ±11.1^{b}	17.6 ±9.8^{abc}	47.9 ±6.5^{ab}
Sample 6	41.8 ±10.8^{a}	42.7 ±8.6^{ab}	47.6 ±2.5^{a}	51.1 ±8.8^{b}	52.8 ±15.0^{bc}	45.1 ±20.9^{ab}
Sample 7	47.1 ±3.4^{a}	36.0 ±2.6^{ab}	47.7 ±8.6^{a}	54.5 ±20.2^{b}	51.6 ±11.9^{bc}	38.1 ±7.2^{ab}
Sample 8	42.7 ±5.1^{a}	29.6 ±4.5^{b}	43.9 ±4.6^{a}	48.6 ±12.0^{b}	71.3 ±6.3^{ab}	34.8 ±2.1^{b}
Sample 9	40.9 ±7.4^{a}	38.5 ±8.9^{ab}	38.7 ±8.0	60.5 ±18.7^{ab}	79.2 ±3.5^{a}	55.7 ±12.6^{ab}
Sample 10	51.7 ±5.8^{a}	41.2 ±9.4^{ab}	41.9 ±6.5^{a}	52.7 ±10.2^{b}	64.9 ±9.2^{ab}	47.4 ±9.2^{ab}
Sample 11	48.7 ±9.0^{a}	49.6 ±6.7^{a}	41.4 ±8.5^{a}	58.3 ±12.2^{b}	66.6 ±15.9^{ab}	55.8 ±7.3^{ab}

Sample 12	48.5 ±5.2^{a}	43.4 ±11.9^{ab}	36.6 ±18.6^{a}	53.9 ±11.3^{b}	54.5 ±7.8^{bc}	59.5 ±11.7^{a}

Values are means of five replicates ± SD.

Different letters in each column represent significant different.

Retention%=Carotenoids content after digestion/ carotenoids content before digestion

Table 4.5 DPPH radical scavenging capacity of whole digesta in different processed foods after digestion (µmol Trolox equiv./g DW)

	Raw	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	702.7 ±71.6^{abc}	512.4 ±46.3^{abc}	516.4 ±89.0^{bcd}	483.7 ±59.3^{a}	154.9 ±98.6^{c}	495.8 ±33.5^{ab}
Sample 2	791.1 ±101.2^{abc}	553.8 ±35.2^{a}	553.5 ±63.2^{abc}	577.3 ±59.5^{a}	457.1 ±128.6^{a}	381.9 ±40.8^{cd}
Sample 3	703.9 ±66.7^{abc}	516.2 ±83.5^{ab}	584.8 ±65.0^{abc}	581.1 ±48.2^{a}	402.6 ±68.6^{ab}	480.6 ±46.0^{ab}
Sample 4	817.6 ±27.7^{ab}	512.7 ±144.9^{abc}	522.3 ±43.7^{abc}	589.6 ±85.1^{a}	312.8 ±81.4^{abc}	538.2 ±29.8^{a}
Sample 5	664.3 ±45.8^{c}	433.3 ±16.4^{bcd}	467.9 ±48.^{d}	490.0 ±71.5^{a}	251.2 ±80.0^{bc}	379.1 ±20.7^{cd}
Sample 6	801.1 ±118.2^{abc}	403.3 ±22.0^{cd}	480.1 ±66.2^{d}	563.8 ±76.2^{a}	411.8 ±78.4^{ab}	467.1 ±39.1^{abc}
Sample 7	776.8 ±21.7^{abc}	475.1 ±35.3^{bcd}	596.0 ±28.6^{a}	641.1 ±115.7^{a}	463.3 ±70.2^{a}	370.8 ±20.4^{d}
Sample 8	675.2 ±31.9^{bc}	446.7 ±22.6^{bcd}	590.1 ±47.7^{ab}	593.2 ±88.2^{a}	400.7 ±63.0^{ab}	365.9 ±41.4^{d}
Sample 9	815.3 ±12.3^{ab}	473.3 ±32.8^{bcd}	532.9 ±33.0^{abc}	488.6 ±78.3^{a}	306.7 ±58.9^{abc}	467.2 ±66.2^{abc}
Sample 10	835.6 ±46.7^{a}	418.3 ±34.2^{bcd}	485.9 ±19.1^{cd}	557.3 ±44.2^{a}	365.2 ±72.5^{ab}	477.8 ±84.5^{ab}
Sample 11	804.8 ±118.4^{abc}	403.2 ±32.4^{cd}	491.7 ±29.1^{bcd}	637.7 ±91.7^{a}	317.9 ±65.7^{ab}	436.9 ±46.9^{bcd}
Sample 12	696.8 ±92.3^{abc}	388.4 ±26.4^{d}	506.1 ±44.9^{bcd}	624.7 ±116.3^{a}	384.3 ±42.5^{ab}	424.3 ±42.0^{bcd}

Table 4.6 DPPH radical scavenging capacity of liquid digesta in different processed foods after digestion (μmol Trolox equiv./g DW)

	Raw	Boiled Maize	Roasted Maize	Fermented Maize	Fermented Porridge	Unfermented Porridge
Sample 1	303.4 ±17.0^{b}	285.2 ±61.1^{ab}	308.3 ±32.6^{a}	348.1 ±44.1^{a}	188.8 ±27.7^{d}	365.0 ±30.7^{a}
Sample 2	315.8 ±44.2^{ab}	294.6 ±10.8^{ab}	303.9 ±16.2^{a}	352.6 ±23.0^{a}	223.1 ±13.8^{d}	336.8 ±38.5^{a}
Sample 3	335.1 ±22.5^{ab}	300.2 ±10.5^{a}	304.3 ±15.5^{a}	395.1 ±20.0^{a}	210.5 ±29.4^{d}	332.4 ±37.6^{a}
Sample 4	316.5 ±17.8^{ab}	293.7 ±10.6^{ab}	301.6 ±53.0^{a}	364.6 ±18.6^{a}	203.7 ±19.8^{cd}	385.6 ±39.2^{a}
Sample 5	302.4 ±31.8^{b}	239.3 ±14.3^{bc}	304.2 ±20.9^{a}	363.8 ±15.6^{a}	225.2 ±23.8^{abc}	439.6 ±17.4^{a}
Sample 6	354.3 ±18.9^{a}	239.6 ±27.4^{bc}	304.1 ±13.0^{a}	330.7 ±22.4^{a}	309.6 ±24.6^{d}	374.4 ±29.5^{a}
Sample 7	299.3 ±17.0^{b}	264.0 ±6.6^{abc}	321.9 ±27.3^{a}	342.0 ±13.5^{a}	295.0 ±11.7^{bc}	356.6 ±18.8^{a}
Sample 8	292.8 ±18.2^{b}	262.1 ±18.0^{abc}	309.4 ±34.2^{a}	353.6 ±21.8^{a}	292.1 ±18.1^{ab}	329.4 ±17.5^{a}
Sample 9	318.9 ±19.0^{ab}	263.5 ±15.5^{abc}	271.5 ±18.6^{a}	336.6 ±14.2^{a}	307.5 ±20.0^{ab}	439.6 ±13.1^{a}
Sample 10	329.6 ±29.7^{ab}	276.7 ±3.8^{abc}	268.3 ±13.7^{a}	341.5 ±17.1^{a}	317.5 ±20.9^{ab}	396.7 ±21.7^{a}
Sample 11	323.0 ±14.7^{ab}	273.0 ±8.1^{abc}	250.4 ±15.3^{a}	374.4 ±15.0^{a}	364.8 ±24.0^{a}	374.6 ±13.4^{a}

Sample 12	327.1±20.1^{ab}	226.0±20.6^{c}	290.4±16.2^{a}	337.7±16.2^{a}	323.2±35.2^{ab}	321.9±24.2^{a}

Values are means of five replicates ± SD.

Different letters in each column represent significant different.

Retention%=Carotenoids content after digestion/ carotenoids content before digestion

5. GENERAL CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

In the present study, the effects of cooking treatments on carotenoids and antioxidant capacity for maize products and the effects of digestion on five different maize-based products (boiled maize, roasted maize, fermented maize, and fermented and unfermented porridge) were analyzed. Results were compared with raw maize samples, which were used as a control. For carotenoids analysis, five carotenoids standards were used: lutein, zeaxanthin, β–cryptoxanthin, α-carotene, and β-carotene. Oxygen radical absorbance capacity (ORAC) and DPPH Radical Scavenging Capacity were used for antioxidant capacity.

Before studying the effects of processing and digestion, the genetic diversity of 12 maize samples was investigated. The 12 samples came from the same original genotype, Mthikinya (MW5021), after harvesting from different geographic and environmental conditions. Yet, they presented different characteristics. This finding could help us understand the differences and similarities of samples following treatments.

Overall, after cooking treatments, all carotenoids had different levels of decrease. The ranking of the processed sample groups was boiled maize (79%), fermented porridge (73.8%), fermented maize (68.4%), unfermented porridge (59.0%), and roasted maize (56.1%). After treatments, the oxygen radical absorbance capacity (ORAC) in all samples remained at the same level or increased. The radical scavenging capacities (DPPH) all decreased, and boiled maize had the highest retention rate of 90.7%. More carotenoids in food products means more carotenoids are available to absorbed and utilized for our body.

After digestion, carotenoids in the whole digesta of raw maize were reduced by 13.4%. However, in boiled, roasted, fermented porridge, and fermented maize, carotenoids in the whole digesta increased compared to the food matrix. The oxygen radical absorbance capacity (ORAC) and radical scavenging capacity activity (DPPH) both had a significant reduction. After investigating the genetic diversity of these 12 maize samples, all 12 showed different characteristics and could be grouped into three sections, which reflect the changes of carotenoids during different treatments. However, during digestion, genetic diversity showed less impact on carotenoids changes.

Based on the results obtained from the present study, maize can provide enough carotenoids and meet the recommended dietary allowance of Vitamin A after being processed into food products. However, there are still additional investigations required to analyze how carotenoids perform after their uptake by the small intestine. Also, during digestion, carotenoids levels change; during this time, some new compounds may have been generated due to the enzymatic reaction. In the future, further HPLC-MS/MS analyses are needed for indicating possible new substances.

Furthermore, more cell lines and cellular-based assays are useful in the investigation of carotenoids transfer and absorption mechanisms in cell culture assays.

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Appendices

Appendix I Genetic analysis of maize samples using microsatellite markers

Abstract

Maize-based products have long been a major source of energy and nutrients, covering over 7% of daily calorie intake all over the world. The objective of this chapter was to evaluate the generic diversity of maize genotype, with Mthikinya (MW5021) as the source of 12 samples used for this study. The genetic diversity of 12 maize samples was estimated by microsatellite markers. A dendrogram was generated using the un-weighted pair-group method with arithmetic means (UPGMA), based on Nei’s genetic distance in Power Marker 3.25. The similarities and differences of allelic loci for maize was based on 40 simple sequence repeats (SSR) markers. Three major clusters were observed and three groups could be identified. Since the maize samples were all grown in different geographic environments and open pollinated and therefore potentially products of cross pollination, the seeds were likely to be contaminated.

Introduction

In eastern and southern Africa, maize-based products have been a major source of energy and nutrients for human consumers, providing over their 70% of daily calorie intake (Ecker & Qaim, 2011). Since 2005, the Malawi government planned to increase maize production. As a result, the maize yield was improved by 2.9 times in 2014 (Murayama et al., 2017). Maize is commonly classified into local and hybrid varieties in Malawi. According to Heisey and Small (1995), varieties not directly coming from the national agricultural research systems are defined as local. For hybrid varieties, seeds are improved through hybridization or open-pollination. In this project, Mthikinya (MW5021) was provided to all 12 villages, and samples were collected after harvest. The genetic diversity of the 12 maize samples was estimated by microsatellite markers. Microsatellites are tracts of repeatable DNA sequences. They can be referred to as simple sequence repeats (SSR) and are widely used in DNA profiling. Some methods have been devised, such as the polymerase chain reaction (PCR), where DNA fragments are amplified and compared with standard SSR markers for maize (Ahmed et al., 2018). The aim was to understand the extent to which the various environments could affect genetic diversity from the same original maize variety.

Materials and Methods

Sample information

A total of 12 maize samples, designated as Sample 1 to Sample 12, were harvested in 2014 from central and northern regions of Malawi, specifically in the Lobi and Mzimba districts. These landrace orange maize seeds were from one variety cultivated in Malawi, named Mthikinya (MW5021). The seeds were distributed and cultivated in different villages. Since the maize plants were open pollinated, cross pollination was likely to occur. These maize samples were shipped from Malawi to the University of Manitoba, sealed in clear Whirlpak plastic sample bags, and kept under -40℃ prior to analysis.

DNA Isolation and genotyping

Five seeds of each accession (maize samples, in this case) were germinated for DNA extraction. Genomic DNA was extracted using the CTAB method (Doyle 1991). The primer sequences of SSR markers are listed in Table A:

Supplementary Table A The primer sequences of SSR markers

code	Accession name	code	Accession name
Y01	Sample 12	Y07	Sample 1
Y02	Sample 11	Y08	Sample 7
Y03	Sample 9	Y09	Sample 4
Y04	Sample 10	Y10	Sample 3
Y05	Sample 5	Y11	Sample 6
Y06	Sample 8	Y12	Sample 2

A PCR reaction was performed in a 10 μl reaction mixture containing 30 ng of template DNA, 1X PCR buffer, 2mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of each primer, and 1 unit of Taq DNA polymerase. All amplifications were performed on MyGene^{TM} Series Peltier Thermal Cycler (Model MG96G) under the following conditions: 5 min at 94℃, followed by 30s at 94℃, 58s at TA, 35s at 72℃ for 35 cycles, and then 7 min at 72℃ for a final extension. The PCR products were separated on 8% polyacrylamide gels and visualized by silver staining.

Due to potential admixture in the maize accessions, the SSR amplification bands were scored 1 for presence and 0 for absence at the same mobility, forming the original matrix. A dendrogram was generated using the un-weighted pair-group method with arithmetic means (UPGMA), based on Nei's genetic distance in Power

Marker 3.25 (Nei, Tajima, & Tateno, 1983). The cluster image was viewed in MEGA8.0.

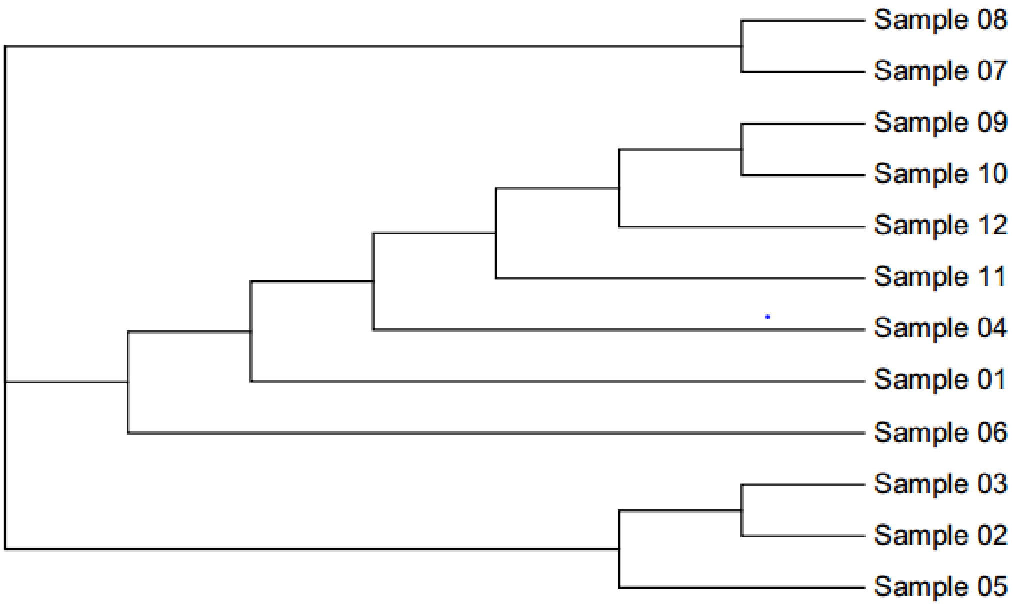
Statistical analysis

All the analyses were carried out at least in duplicate. Data analyses were conducted using SPSS statistics 19.0 (IBM). Analysis of variance (ANOVA) and Duncan’s multiple range test were used to compare the significant differences among the means of different accessions. Pearson correlation coefficients were determined to evaluate the relationship between variables. The hierarchical cluster analysis was computed using Ward’s method (Bao, Xing, Phillips, & Corke, 2003).

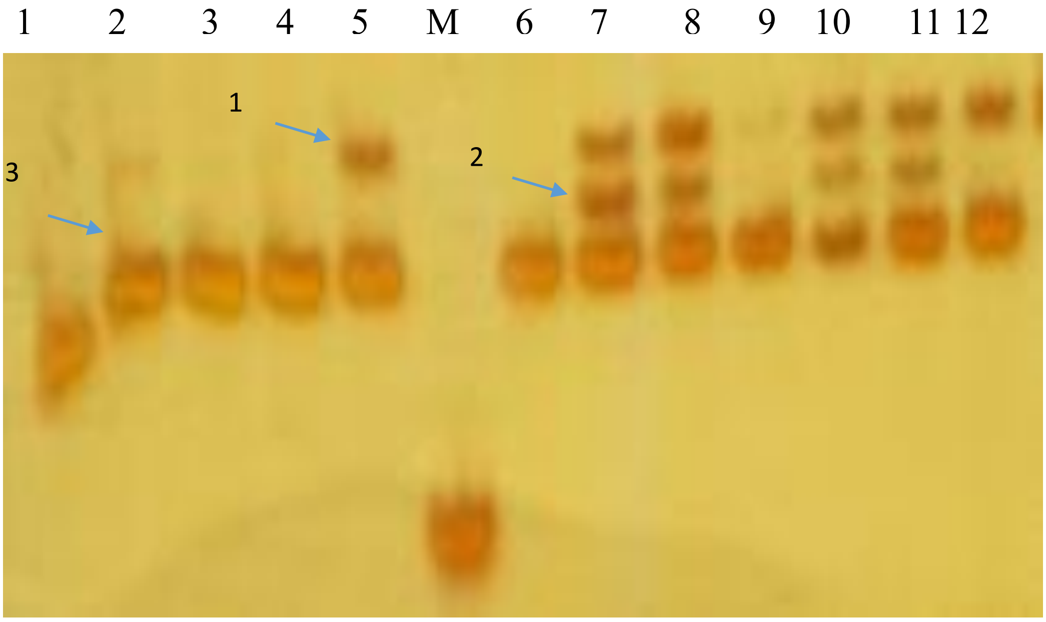
Results and Discussion

The UPGMA dendrogram of 12 maize accessions is shown in Figure A. The similarity and difference in allelic loci for maize were based on 40 SSR markers. Alleles are variants from a given gene and loci is a fixed position on a chromosome. When we compare allelic loci with standard microsatellite markers (SSR markers), and if they exhibit similar performance, we can say the two maize accessions are similar (Wood, 1995). From the figure, three major clusters (groups) were observed, one consisting of 7 accessions defined as Group 2 (including samples 01, 04, 06, 09, 10, 11, and 12), another consisting of 3 accessions defined as Group 3 (including samples 02, 03, and 05), and the last defined as Group 1 consisting of 2 accessions (including samples 07 and 08).

The maize accessions in this study showed admixture in genetic nature, which means that the seeds in each accession contain different alleles. For a pure diploid species, the genotype is homozygous with one allele. Figure B is provided as an example, using the marker YM65. In this case, we found three genotypes, genotype 1, genotype 2, and genotype 3, as marked in Figure 3.2. The lines 2-4 are homozygous with only genotype 3, which means they are pure diploid species. The lines 5 and 12 are heterozygous with genotypes 1 and 3. However, lines 7, 8, 10, and 11 have three alleles, which should be impossible for diploid crops like maize in a single seed. The only possibility is that outcrossing happens during plant growth. In this case, the mother plant was supposed to have only genotype 3 because the band density was higher than the other two bands; however, the pollens came from other accessions with genotypes 1 and 2. Maize is a typical outcrossing species and its seeds are very likely to become contaminated if they are grown in an open field.



Supplementary Figure A. The UPGMA dendrogram of 12 maize accessions based on 40 SSR markers

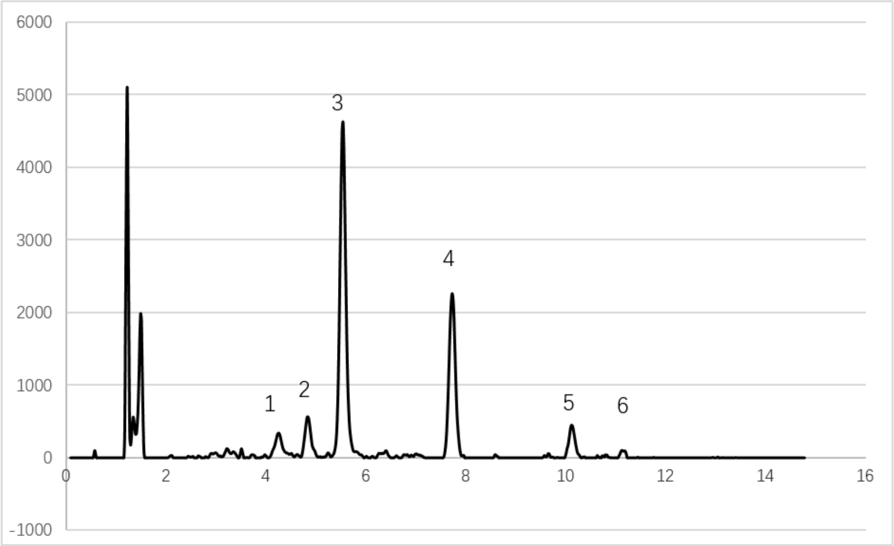


Supplementary Figure B. The PCR product of YM65 SSR marker showing different alleles in maize accessions.

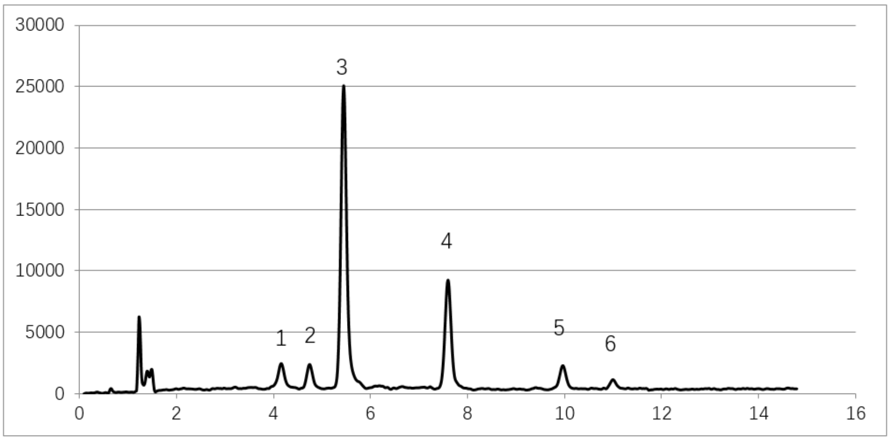
Conclusion

In this study, we investigated the genetic diversity of 12 maize samples. The genetic divergence among maize accessions was determined by using 40 SSR markers. After growing in different geographic and environmental conditions, 12 samples represented different characteristics, even though they were from the same original genotype Mthikinya (MW5021). This finding could help us understand the differences and similarities between samples following treatments.

Appendix II: HPLC chromatograms of carotenoids



A) Standard mixture



B) Raw maize sample

Supplementary Figure C. HPLC chromatograms (at 450 nm) of carotenoids separated from standard mixture (A) and raw maize sample (B); 1, lutein; 2, zeaxanthin; 3, internal standard; 4, β-cryptoxanthin; 5, β-carotene; 6, α-carotene

Appendix III: Tukey-Kramer Grouping for Lutein and Zeaxanthin retention rate

Maize-based Products	**Carotenoid**		
Boiled Maize	lutein		A
	zeaxanthin	B	A
Fermented Porridge	lutein	B	A
	zeaxanthin	B	A
Fermented Maize	lutein	B	A
	zeaxanthin	B	A
Unfermented Porridge	lutein	B	
	zeaxanthin	B	
Roasted Maize	lutein	B	
	zeaxanthin	B	

Least Squares means (Alpha=0.05) with the same letter are not significantly different.