Sorghum pericarp pigments are associated with the contents of carotenoids and provitamin A

by

Yanting Shen

B.S., Kansas State University, 2014

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Dr. Weiqun Wang

Copyright

Abstract

Sorghum is a staple crop consumed in certain regions of Africa and Asia, where vitamin A deficiency is prevalent. However, the correlation of sorghum intake and vitamin A deficiency is inconsistent. The objective of this study was to identify and quantify the carotenoids and pro-vitamin A in the selected sorghum accessions with various pericarp pigments by using LC-MS. Among of total five carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin) that were identified and quantitated, three (α-carotene, β-carotene and β-cryptoxanthin) are precursors of vitamin A. The highest content of total carotenoids was detected in the sorghum accessions with yellow pericarp (PI656096, PI585374, PI563448, PI585351), while the highest β-carotene content was found in the accessions with brown or yellow pericarp (PI655996, PI656096, PI585374, PI563448, PI585351). The lowest carotenoids were found in the accessions with white pericarp (PI533943, PI656112, PI565121, PI560493). The pro-vitamin A was 584.9 ± 38.9 ng/g DW in yellow pericarp, 250.6 ± 28.9 ng/g DW in brown pericarp, and 89.0 ± 12.3 ng/g DW in white pericarp, respectively. It appeared the phenotypic diversity of sorghum pericarp colors was strongly associated with the contents of carotenoids and pro-vitamin A, indicating a different impact of various sorghum varieties on vitamin A deficiency and suggesting a possible prevention of vitamin A deficiency by breeding certain sorghum varieties with pericarp pigments.

Table of Contents

List of Figures

Acknowledgements

Firstly, I would like to express my sincere gratitude to my major advisor Dr. Weiqun Wang for his continuous support during my whole M.S. study research work. His patience and support helped me to overcome many difficulties, crisis situations and this thesis.

I would like to thank my committee members, Dr. J. Scott Smith and Dr. Weixing Song for all their guidance and encouragement through whole process.

I would like to thank Dr. Davina Rhodes and Dr. Tom Herald for providing sorghum grain samples and thank Dr. J. Scott Smith for borrowing C30 column from his lab.

I would like to thank all the graduate students in Dr. Wang's lab. I cannot finish my experiment smoothly without their care and assistance.

Finally, I would especially like to thank my parents and my husband for their love, support and constant encouragement throughout my years of study. This accomplishment would not have been possible without their endless support. Thank you!

Dedication

Dedicated my thesis to my parents and my husband for their endless love, support and encouragement.

Chapter 1 - Literature review

1.1 Sorghum Background:

Sorghum [*Sorghum bicolor (L.) Moench*] is an essential source of food for both human and animals in developing countries. It ranks the fifth most important crop in the world of total production, and it consists of the major source of protein, calories and minerals for more than 500 million people, especially in Southern Asia and Africa[1]. More than 35% of sorghum is grown directly for human consumption, and the rest is used for animal feed, alcohol and industrial products. A majority of population in Africa and central India depends on sorghum for their dietary energy and micronutrient requirements[1–3]. United State is the largest producer and exporter of sorghum, accounting for 17% of world production and almost 75% of world sorghum exports in 2014-2015[4]. In Africa, the most important cereal is maize, followed by sorghum, and the average of sorghum consumption is per capita of 23 kg/person/year[5]. Nigeria is the largest sorghum producer in Africa, and it accounts about 35% of the African production[2]. According to World Health Organization, there were estimated 75-140 million children to be vitamin A deficiency, and 4.4 million children have xerophthalmia and more than 6 million women develop night blindness during pregnancy every year[6,7]. Approximately 45% of vitamin A deficiency children and pregnant women live in South and Southeast Asia[6]. It is important to know that vitamin A deficiency is worldwide distributed, especially in high sorghum consumption areas[7]. Sorghum is an important source of bioactive compound including various phytochemicals such as phenolic compounds, tannins, anthocyanins, carotenoids and other antioxidants[3]. These phytochemicals reduce the damage caused by free radicals and promote benefits to human health[8].

1.2 Phenotypic Sorghum

The association of sorghum intake and vitamin A deficiency is not fully supported by several studies. Some studies indicated that sorghum as the main diet in most of Africa will induce the various types of malnutrition such as vitamin A deficiency [9,10]. Other studies have linked that sorghum in Africa and Asia, is a critical source of dietary phytochemicals including carotenoid that might prevent vitamin A deficiency[11,12]. Based on different sorghum varieties **(Fig.1.1.)**, result for the association of sorghum and vitamin A deficiency is inconclusive. The sorghum grain consists of pericarp (outer covering), testa (layer between the pericarp and endosperm), endosperm (storage tissue), and germ (embryo)[13]. The pericarp originally from the ovary wall, and it can divided into four parts, which are the epicarp, the mesocarp, the cross-cell layer, and the tube cell layer[14]. Epidermis, which is the first cell layer contains the most pigments in the pericarp. There are several factors that will influence the color of sorghum grains by eyes, for example: the genetics of pericarp color, pericarp thickness, color of the testa and the endosperm color. There are also some genes control the pericarp color during the polyphenol biosynthesis. For example, R-Ygenes determine the pericarp color is red (R-Y-), colorless or white (r-YY, RRYY) or yellow (rrY-). The intensifier gene (I-) affects the intensity of the pericarp color when R-Y-gene are present. Genotypes with dominant allele at the spreader (S) locus, as well as B1and B2 loci will result in a brown color appearance of sorghum grain[13,15].

Figure 1.1 Picture of various pigmented pericarp of sorghum

1.3 Vitamin A

Carotenoids can be divided into provitamin A and non-provitamin A compounds. According to Figure 1, the major provitamin A carotenoid including β-carotene, α-carotene and β-cryptoxanthin, and they have potential health benefit to prevent vitamin A deficiency[7]. Vitamin A is essential for human health, and it can be obtained from food, either as vitamin A in animal products, such as eggs and dairy products, or provitamin A from plant origin such as fruit and vegetables[16]. Vitamin A needed in infants, children, elderly, pregnant and lactating women. The recommended dietary intake (DRI) for men and women are 700μg and 600μg, respectively[17]. In developing countries especially Africa and South-East Asia, the consumption of vitamin A is much less. As a result of that, vitamin A deficiency causes many visual defects, immune system problems, blindness, growth retardation, and severity of disease[7,18–20]. The conversion of dietary β-carotene to vitamin A is not efficient by dietary sources. In humans, conversion of β-carotene to vitamin A takes place in the intestine[16]. Conversion of β-carotene to vitamin A cleaves the central double bond of β-carotene molecule to yield two 20-carbon molecules of vitamin A theoretically, while α-carotene is not symmetrical like β-carotene, therefore it only can convert to one molecule of vitamin A. So α-carotene is as half efficient as β-carotene in producing vitamin A in human body[21]. In fact, lower conversion rate is reported that dietary β-carotene to vitamin A is only 12:1 by weight, α-carotene and β-cryptoxanthin are both 24:1 of vitamin A[16,22]. The major factors that affect the bioavailability and bioconversion of food carotenoid from provitamin A are food preparation, food matrices properties, and the content of fat[16,19].

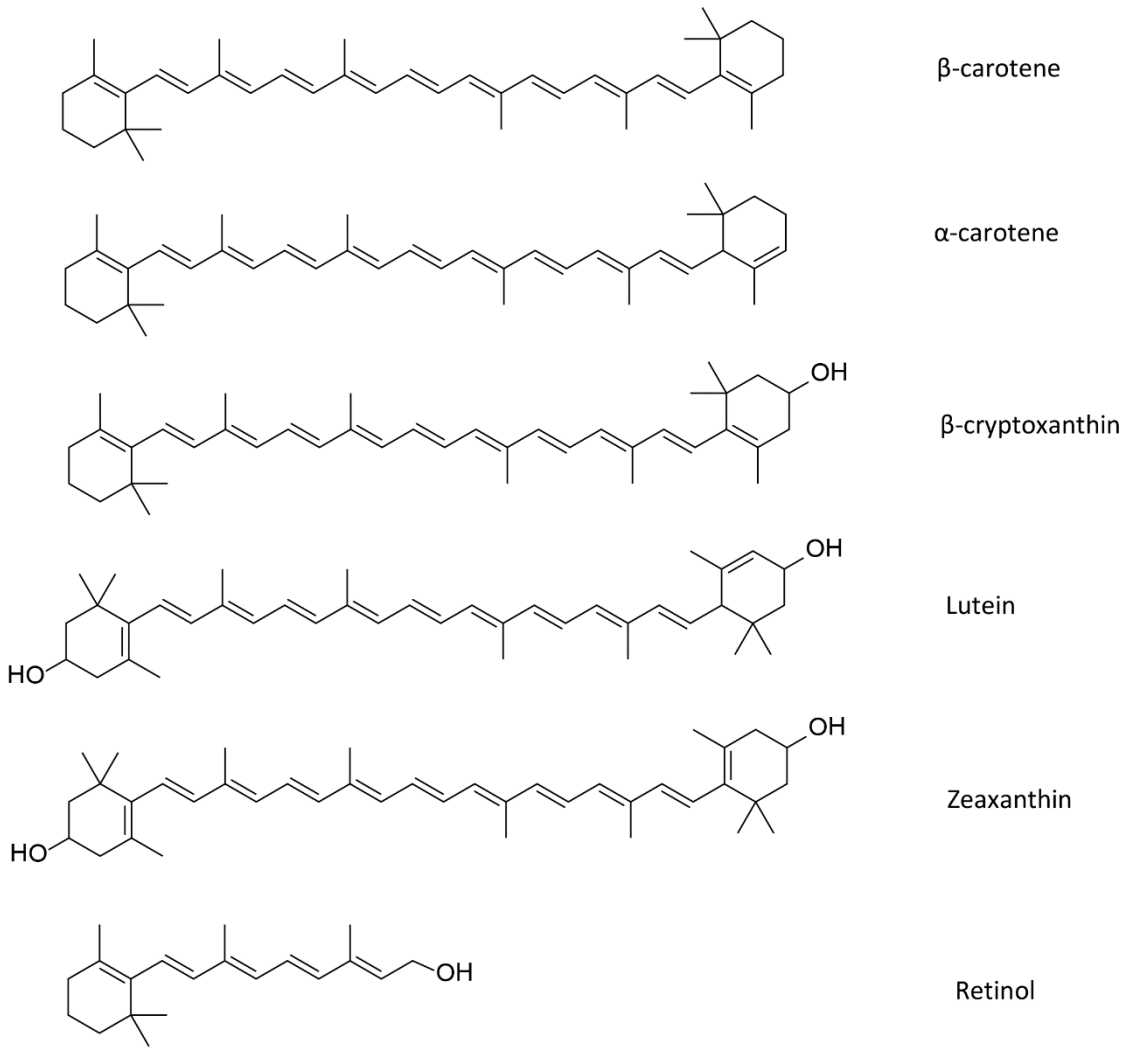

Figure 1.2 Chemical structures of the common carotenoids found in sorghum versus vitamin A retinol

1.4 Carotenoids

Carotenoids are pigmented compound that are synthesized by plants and microorganisms, not humans or animals. The function of carotenoid in photosynthesis is to protect against photo-damage[19]. Fruits and vegetables are major food sources of carotenoids in human diet, and they express yellow, red, and orange colors[19,23]. Carotenoids have beneficial properties of preventing

human disease including cardiovascular disease, cancer, cataracts and other chronic disease[24]. Carotenoids are important dietary sources of vitamin A[25]. According to their chemical composition in Figure 1, they have two categories, which are carotenes and xanthophyll. β-carotene, α-carotene and lycopene are prominent of carotene group, which contain only carbon and hydrogen atoms, while xanthophylls consist of at least one hydrogen atom, such as lutein, zeaxanthin[19,25,26], and they are more polar than carotene[27]. Because of the number of double bonds of carotenoids chemical structure, there have *cis/trans* configurations. Generally, trans configuration of carotenoid is thermodynamically stable in nature, while *cis* isomers are present in blood and tissue[23]. Most of the carotenoids in the diet and human body are represented as β-carotene, α-carotene, lycopene, lutein and cryptoxanthin[23].

1.5 Carotenoids and Disease

According to the World Health Organization, cancer is the main cause of death and it accounts for 8.2 million of all death in 2012[28]. Therefore, it is important be aware of and reduce the death rate of cancer development. A number of studies have shown that increased intake fruit and vegetables that rich in bioactive compound will decrease the risk of several type of cancer disease.

Lutein and zeaxanthin are only carotenoids that found in retina. Evidence from human studies suggested that dietary intake of these two antioxidant nutrients play an important role in the protection against eye disease such as age-related macular degeneration (AMD)[29,30]. According to NIH National Eye Institute, there is a nutritional supplement called AREDS formulation could reduce the risk of AMD. The original formulation contains β-carotene, because β-carotene may increase the risk of lung cancer among people whom smoke, so they

used lutein and zeaxanthin instead of β-carotene as new formulation AREDS2[31–33]. Lutein and zeaxanthin may be safer and more effective than β-carotene. Similar study from Landrum[34], found that intake supplement with lutein (30mg/day for 140days) resulted in increase serum levels of lutein as well as increase in the lutein of macula on human eye.

Lycopene is a powerful antioxidant because of its multiplicity of conjugated double bonds, and it prevents free radicals. Large epidemiological studies have shown that people who eat tomato lead to reduce the risk of cancer. In the United States, approximately 9% of cancer death among men belongs to prostate cancer in 2006[24]. As reviewed from epidemiological studies that have showed whether carotenoids or foods that contains high levels of carotenoids have potential protective effect of prostate cancer. The richest source in typical diet is tomato or tomato related food products[24]. An observational study that contains 11 case-control and 10 Cohot studies indicated that approximately 10-20% reduction in risk of prostate cancer that associated with high and low intake of tomatoes[24]. For dietary factors, high tomato consumptions of 5 or more per week was associated with decrease risk of cancer[26].

Although β-carotene is one of the provitamin A, there is a side effect of taking β-carotene as the dietary supplement. Lung cancer is one of the top fatal cancer diseases in United State for both men and women with the major risk factor being smoking. In early 1990s, there was strong evidence to indicate that increase consumption of β-carotene rich fruit or vegetables will decrease lung cancer[35–37]. Recently, large number of review and meta-analysis found that high dose of β-carotene supplementation about 20-30 mg/day is associated with increase lung cancer in smokers and asbestos works due to increased oxidative stress, altered retinoic acid signaling, and cytochrome P450 induction. However, low dose of β-carotene supplement or dietary β-carotene food product are more protective than harmful to lung cancer development[35,36].

1.6 Analysis of Carotenoids in Nutritional Studies

1.6.1 Extraction

Extraction method is critical to recovery of antioxidant phytochemicals. The natural of plant materials and bioactive compound should be considered to achieve good separation and extraction efficiency. A variety of organic solvent could be used in extraction, including acetone, tetrahydrofuran (THF), ethyl acetate, petroleum ether/methanol, or hexane/methanol[38,39]. Ellie's method for carotenoids extraction of sorghum is to use saponification with 30% methanolic NaOH for 30 min in dark, and carotenoids are extracted with petroleum ether/acetone (3:1), containing 0.1% BHT to prevent the formation of peroxide which could lead carotenoids degradation[12]. Another study indicated that carotenoids from sorghum can be extracted by twice with cold acetone and once with methyl *tert*-butyl ether without saponification[10]. However, saponification is a critical step during the extraction of carotenoids involved with alcoholic potassium or sodium hydroxide. Alkaline hydrolysis can be used to remove interfering lipid and chlorophylls present in plant and food samples. Saponification also hydrolyzes carotenol fatty acyl esters to simplify chromatographic separation.

1.6.2 HPLC separation of Carotenoids

HPLC separation method is essential to achieve the success of identification of carotenoids. Ellie[12] was used Hewlett-Packard model 1090A HPLC system equipped with diode array detector. Carotenoids separation was achieved by using reversed-phase (4.6×250mm) polymeric C18 column with a guard column. A gradient elution was conducted by using methanol/1M ammonium acetate (98:2 v/v) in phase A and ethyl acetate in phase B. A flow rate

was set at 1.0 ml/min with the initial concentration at 100%A and gradient to 80:20 A/B though 20 minutes. The gradient was hold for 5 minutes at the highest concentration, and another 5 minutes back to the initial condition. The other study indicated that using the same column, but isocratic elution, which mobile phase consists of hexane/isopropanol (95:5, v/v), and the flow rate is 1.3 ml/min[40]. Another study used the YMC carotenoid C30 analytical column with mobile phase A was methanol/methyl-tert-butyl-ether/water (81: 15: 4), and mobile phase B was methanol/methyl-tert-butyl-ether (9:91)[41]. Compare to classical C18 stationary phase, YMC Carotenoid C30 is polymeric synthesized with a reversed phase column bonded with C30 chain stationary phase. It is much more hydrophobic than C18 column, and it has higher selectivity to determine the molecular shape and better resolution and separation of different geometrical isomers of carotene identification[42,43]. Trans-β-apo8'-carotenal has been used as an internal standard for quantification of carotenoids in sorghum accessions. All the carotenoids peaks were detected at 450 nm wavelengths.

1.7 Carotenoids contents in sorghum

The color of grain sorghum in endosperm and pericarp is determined by genetic controlling. Endosperm only can exhibit yellow or white color. According to the National Plant Gemplasm System (USDA-ARS), the data showed that only 381 accessions of sorghum exhibited yellow endosperm color from 42,869 of total sorghum (*Sorghum bicolor* Moench) accessions. A set of 289 accessions exhibited as mixed endosperm color, which may include some yellow and some white. In addition, 2,334 accessions of sorghum have white endosperm color[44]. Yellow endosperm sorghum contains higher provitamin A including β-carotene, α-carotene and β-cryptoxanthin, which can potentially prevent vitamin A deficiency[41]. Lutein,

zeaxanthin and β-carotene are three major carotenoid compounds in sorghum accessions[41]. The carotenoids contents of selected yellow endosperm sorghum accessions were reported by Kean. E[45] and Cardoso.is about 0.112-0.351 μg/g DW and 0.0212-0.8546 μg/g DW respectively. Zeaxanthin was the most abundant carotenoids in yellow endosperm sorghum around 0.27 μg/g DW, and α-carotene and β-cryptoxanthin are hard to detect because of very low content[45]. Fernandez. M[13] conducted another experiment to compare the carotenoids content base on endosperm color of sorghum, and the result from his experiment showed that total carotenoid content in yellow and white endosperm are around 1.68 and 0.20 μg/g DW, respectively. As the result of that, there is a correlation between phenotypic value of carotenoid and endosperm color. Yellow color has positively correlated with concentration of all carotenoids.

The positive correlation between carotenoid contents and yellow endosperm sorghum accessions had been established. However, relatively few data have been published regarding the carotenoid contents in various pericarp pigments of sorghum grains. The objective of this study was to identify and quantify the carotenoids and pro-vitamin A in the selected sorghum accessions with various pericarp pigments. The results may suggest a possible prevention of vitamin A deficiency by breeding certain sorghum varieties with pericarp pigments.

1.8 Reference

(1) Pontieri, P.; Troisi, J.; Di Fiore, R.; Di Maro, A.; Bean, S. R.; Tuinstra, M. R.; Roemer, E.; Boffa, A.; Del Giudice, A.; Pizzolante, G.; et al. Mineral contents in grains of seven food-grade sorghum hybrids grown in a Mediterranean environment. *Australian Journal of Crop Science* 2014, *8* (11), pp 1550–1559.

(2) Gourichon H. Analysis of incentives and disincentives for sorghum in Nigeria. Technical notes series, MAFAP, FAO,Rome. 2013.

(3) Awika, J. M.; Rooney, L. W. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*. 2004, pp 1199–1221.

(4) United State Department of Agriculture-Foreign Agriculture Division, 2003 data. Available from: https://apps.fas.usda.gov/psdonline/.

(5) Maunder, B. *Sorghum: The global grain of the future; National grain sorghum producers:Lubbock, TX*; 2005.

(6) West, K. P. Extent of vitamin A deficiency among preschool children and women of reproductive age. *The Journal of nutrition* 2002, *132* (9 Suppl), pp 2857S – 2866S.

(7) Britton, G. Analysis of Carotenoids in Nutritional Studies. In *Carotenoids*; Birkhäuser Basel: Basel, 2009; pp 12–21.

(8) Cardoso, L. de M.; Montini, T. A.; Pinheiro, S. S.; Queiroz, V. A. V.; Pinheiro-Sant'Ana, H. M.; Martino, H. S. D.; Moreira, A. V. B. Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum. *Food chemistry* 2014, *152*, pp 210–217.

(9) Adegboye, O. roseline; Smith, C.; Anang, D.; Musa, H. Comparing and Contrasting Three Cultural Food Customs from Nigeria and Analyzing the Nutrient Content of Diets from

These Cultures with the Aim of Proffering Nutritional Intervention. *Critical Reviews in Food Science and Nutrition* 2016, *56* (15), pp 2483–2494.

(10) Lipkie, T. E.; De Moura, F. F.; Zhao, Z. Y.; Albertsen, M. C.; Che, P.; Glassman, K.; Ferruzzi, M. G. Bioaccessibility of carotenoids from transgenic provitamin a biofortified sorghum. *Journal of Agricultural and Food Chemistry* 2013, *61* (24), pp 5764–5771.

(11) Kean, E. G.; Bordenave, N.; Ejeta, G.; Hamaker, B. R.; Ferruzzi, M. G. Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. *Journal of Cereal Science* 2011, *54* (3), pp 450–459.

(12) Kean, E. G.; Ejeta, G.; Hamaker, B. R.; Ferruzzi, M. G. Characterization of carotenoid pigments in mature and developing kernels of selected yellow-endosperm sorghum varieties. *Journal of agricultural and food chemistry* 2007, *55* (7), pp 2619–2626.

(13) Salas Fernandez, M. G.; Hamblin, M. T.; Li, L.; Rooney, W. L.; Tuinstra, M. R.; Kresovich, S. Quantitative trait loci analysis of endosperm color and carotenoid content in sorghum grain. *Crop Science* 2008, *48* (5), pp 1732–1743.

(14) Rooney, L. W.; Murty, D. S. Variation in the structure and kernel characteristics of sorghum. In International Symposium on Sorghum Grain Quality. 1982, pp 150–176.

(15) Rhodes, D. H.; Hoffmann, L.; Rooney, W. L.; Ramu, P.; Morris, G. P.; Kresovich, S. Genome-wide association study of grain polyphenol concentrations in global sorghum [Sorghum bicolor (L.) Moench] germplasm. *Journal of Agricultural and Food Chemistry* 2014, *62* (45), pp 10916–10927.

(16) Tang, G. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *The American journal of clinical nutrition* 2010, *91* (5), pp 1468S – 1473S.

(17) Olson, J. A. Recommended dietary intakes (RDI) of vitamin A in humans. *American*

Journal of Clinical Nutrition 1987, *45* (4), pp 704–716.

(18) Weber, D.; Grune, T. The contribution of β-carotene to vitamin A supply of humans. *Molecular Nutrition and Food Research*. 2012, pp 251–258.

(19) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacological Research*. 2007, pp 207–216.

(20) Fiedor, J.; Burda, K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients* 2014, *6* (2), pp 466–488.

(21) Graham R, R. J. Carotenoids in staple foods: Their potential to improve human nutrition. *Food and Nutrition Bulletin* 2000, *21* (4), pp 404–409.

(22) Institute of Medicine, F. and N. B. Beta-carotene and other carotenoids. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. *Washington, D.C.: National Academy Press* 2000, pp 325–400.

(23) Paiva, S. a; Russell, R. M. Beta-carotene and other carotenoids as antioxidants. *Journal of the American College of Nutrition* 1999, *18* (5), pp 426–433.

(24) Mayne, S. T.; Wright, M. E.; Cartmel, B. Epidemiology and Intervention Trials. In *Carotenoids*; Birkhäuser Basel: Basel, 2009; pp 191–210.

(25) Stahl, W.; Sies, H. Bioactivity and protective effects of natural carotenoids. In *Biochimica et Biophysica Acta - Molecular Basis of Disease*; 2005; Vol. 1740, pp 101–107.

(26) Johnson, E. J. The role of carotenoids in human health. *Nutrition in clinical care : an official publication of Tufts University* 2002, *5* (2), pp 56–65.

(27) Cuttriss, A. J.; Cazzonelli, C. I.; Wurtzel, E. T.; Pogson, B. J. Carotenoids. *Advances in Botanical Research* 2011, *58*, pp 1–36.

(28) Lindshield, B. L.; King, J. L.; Wyss, A.; Goralczyk, R.; Lu, C.-H.; Ford, N. A.; Erdman, J.

W. Lycopene Biodistribution Is Altered in 15,15'-Carotenoid Monooxygenase Knockout Mice. *Journal of Nutrition* 2008, *138* (12), pp 2367–2371.

(29) Ribaya-Mercado, J. D.; Blumberg, J. B. Lutein and zeaxanthin and their potential roles in disease prevention. *Journal of the American College of Nutrition* 2004, *23* (6 Suppl), pp 567S – 587S.

(30) Ma, L.; Dou, H.-L.; Wu, Y.-Q.; Huang, Y.-M.; Huang, Y.-B.; Xu, X.-R.; Zou, Z.-Y.; Lin, X.-M. Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. *The British journal of nutrition* 2012, *107* (3), pp 350–359.

(31) Age-Related Eye Disease Study Research Group; SanGiovanni, J. P.; Chew, E. Y.; Clemons, T. E.; Ferris, F. L.; Gensler, G.; Lindblad, A. S.; Milton, R. C.; Seddon, J. M.; Sperduto, R. D. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Archives of ophthalmology (Chicago, Ill. : 1960)* 2007, *125* (9), pp 1225–1232.

(32) Clemons, T. E.; Chew, E. Y.; Bressler, S. B.; McBee, W. National Eye Institute Visual Function Questionnaire in the Age-Related Eye Disease Study (AREDS): AREDS Report No. 10. *Archives of ophthalmology* 2003, *121* (2), pp 211–217.

(33) Eye, T. A. AREDS Report No. 8. *Arch Ophthalmol* 2001, *119*, pp 1417–1436.

(34) Landrum, J. T.; Bone, R. a; Joa, H.; Kilburn, M. D.; Moore, L. L.; Sprague, K. E. A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Experimental eye research* 1997, *65* (1), pp 57–62.

(35) Russell, R. M.; Mayer, J. Beta-carotene and lung cancer*. *Pure Appl. Chem* 2002, *74* (8), pp 1461–1467.

(36) Lotan, R. Lung Cancer Promotion by -Carotene and Tobacco Smoke: Relationship to Suppression of Retinoic Acid Receptor- and Increased Activator Protein-1? *JNCI Journal of the National Cancer Institute* 1999, *91* (1), pp 7–9.

(37) Albanes, D. Beta-carotene and lung cancer: a case study. *The American journal of clinical nutrition* 1999, *69* (6), pp 1345S – 1350S.

(38) Khachik, F. Analysis of Carotenoids in Nutritional Studies. In *Carotenoids*; Birkhäuser Basel: Basel, 2009; pp 7–44.

(39) Amorim-Carrilho, K. T.; Cepeda, A.; Fente, C.; Regal, P. Review of methods for analysis of carotenoids. *TrAC - Trends in Analytical Chemistry*. 2014, pp 49–73.

(40) Cardoso, L. D. M.; Pinheiro, S. S.; Da Silva, L. L.; De Menezes, C. B.; De Carvalho, C. W. P.; Tardin, F. D.; Queiroz, V. A. V.; Martino, H. S. D.; Pinheiro-Sant'Ana, H. M. Tocochromanols and carotenoids in sorghum (Sorghum bicolor L.): Diversity and stability to the heat treatment. *Food Chemistry* 2015, *172*, pp 900–908.

(41) Fernandez, M. G. S.; Kapran, I.; Souley, S.; Abdou, M.; Maiga, I. H.; Acharya, C. B.; Hamblin, M. T.; Kresovich, S. Collection and characterization of yellow endosperm sorghums from West Africa for biofortification. *Genetic Resources and Crop Evolution* 2009, *56* (7), pp 991–1000.

(42) Rivera, S. M.; Canela-Garayoa, R. Analytical tools for the analysis of carotenoids in diverse materials. *Journal of Chromatography A*. 2012, pp 1–10.

(43) Rimmer, C. A.; Sander, L. C.; Wise, S. A. Selectivity of long chain stationary phases in reversed phase liquid chromatography. *Analytical and Bioanalytical Chemistry* 2005, *382* (3), pp 698–707.

(44) Salas Fernandez, M. G.; Kapran, I.; Souley, S.; Abdou, M.; Maiga, I. H.; Acharya, C. B.;

Hamblin, M. T.; Kresovich, S. Collection and characterization of yellow endosperm sorghums from West Africa for biofortification. *Genetic Resources and Crop Evolution* 2009, *56* (7), pp 991–1000.

(45) Kean, E. G.; Ejeta, G.; Hamaker, B. R.; Ferruzzi, M. G. Characterization of carotenoid pigments in mature and developing kernels of selected yellow-endosperm sorghum varieties. *Journal of Agricultural and Food Chemistry* 2007, *55* (7), pp 2619–2626.

Chapter 2 - Experiment

2.1 Abstract

Sorghum is a staple crop consumed in certain regions of Africa and Asia, where vitamin A deficiency is prevalent. However, the correlation of sorghum intake and vitamin A deficiency is inconsistent. The objective of this study was to identify and quantify the carotenoids and pro-vitamin A in the selected sorghum accessions with various pericarp pigments by using LC-MS. Among total five carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin) that were identified and quantitated, three (α-carotene, β-carotene and β-cryptoxanthin) are precursors of vitamin A. The highest content of total carotenoids was detected in the sorghum accessions with yellow pericarp (PI656096, PI585374, PI563448, PI585351), while the highest β-carotene content was found in the accessions with brown or yellow pericarp (PI655996, PI656096, PI585374, PI563448, PI585351). The lowest carotenoids were found in the accessions with white pericarp (PI533943, PI656112, PI565121, PI560493). The pro-vitamin A was 584.9 ± 38.9 ng/g DW in yellow pericarp, 250.6 ± 28.9 ng/g DW in brown pericarp, and 89.0 ± 12.3 ng/g DW in white pericarp, respectively. It appeared the phenotypic diversity of sorghum pericarp colors was associated with the contents of carotenoids and pro-vitamin A, indicating a different impact of various sorghum varieties on vitamin A deficiency and suggesting a possible prevention of vitamin A deficiency by breeding certain sorghum varieties with pericarp pigments.

Key words: carotenoids; pro-vitamin A; sorghum; pericarp pigment; vitamin A deficiency.

2.2 Introduction

Grain sorghum (*Sorghum bicolor* Moench) is a staple food for both human and animals in certain areas such as Southern Asia and Africa where the climate is too hot and dry for other grains [1,2]. As the 2nd most important cereal, the average of sorghum consumption per capita in Africa is 23 kg/person/year [2]. United States is the largest producer in the world of sorghum production in 2014-2015, and then followed by Nigeria, Sudan, Mexico and India [2]. Because of the high sorghum production in Africa and Asia, people usually make sorghum as tortilla, porridge, couscous[3,4]. Sorghum as the main dietary food, it has advantages and disadvantages. For its advantages, sorghum contains anthocyanins and phenolic acid that distributed in the sorghum bran have antioxidant activity to prevent chronic disease, for example, cardiovascular or certain cancer disease[5]. Sorghum is a gluten free food product, so it is safe to consume for whom have celiac disease[6]. In addition, sorghum contains high content of fiber, and could be lower the glycemic index that could help to prevent obesity and diabetes[4]. On the other hand, sorghum has poor sensory quality and hard to digest due to the protein crosslinking properties[7]. Some sorghum contains tannins, so that could be difficult for human body to absorb other nutrients[5,7]. Therefore, United States usually use sorghum as animal feed[1]. According to the World Health Organization, there were estimated 75-140 million children with vitamin A deficiency. Among this group 4.4 million of children developed the symptoms of xerophtalmia, and 6 million pregnant women had night blindness during pregnancies every year [8]. In South and Southeast Asia, there are about 45% of the population of children and pregnant women with vitamin A deficiency. In Africa, more than half of this same population are affected [9]. We surmise that the severity of vitamin A deficiency in these populations is the result of consuming sorghum varieties low in vitamin A precursors.

The source of vitamin A that can be found in animal origin like liver, milk and eggs, etc. Carotenoids are one of the natural pigments and phytochemicals that can be found in plants, fruits and green leaf vegetables, including carotenes (β-carotene, α-carotene, and lycopene) and xanthophyll (lutein, zeaxanthin, and β-cryptoxanthin). As shown in Figure2.2, some chemical structures of common carotenoids including β-carotene, α-carotene, and β-cryptoxanthin are compatible with retinol structure, and thus have nutritional value of provitamin A [10–13]. Conversion of β-carotene to vitamin A involves cleavage of the central double bond of β-carotene molecule to yield two 20-carbon molecules of vitamin A. In contrast, α-carotene or β-cryptoxanthin generates just one molecule of retinol. So α-carotene or β-cryptoxanthin yields just half of the vitamin A compared to β-carotene [14]. In fact, much lower conversion rates are reported for the conversion of dietary β-carotene to vitamin A, just 12:1 by weight, and just 24:1 for α-carotene and β-cryptoxanthin [15,16]. The Recommended dietary intake (RDI) of vitamin A for women and men is 700 μg and 600 μg respectively[17]. Vitamin A is needed for infant, children, the elderly, pregnant and lactating women. Vitamin A deficiency will causes many problems, for example: immune system problem, malformation during embryogenesis, growth retardation and chronic disease[17–19].

The association of sorghum intake and vitamin A deficiency is not fully supported by published studies. Some studies looking at Africans have indicated that when sorghum is the principle grain in the diet that various types of malnutrition including vitamin A deficiency are observed [20,21]. Other studies suggested that the sorghum consumed in Africa and Asia could be a critical source of dietary carotenoids that might provide the needed pro-vitamin A's [22,23]. These inconsistent conclusions may be related to the differences in the sorghum varieties consumed.

These varieties could vary in the colors of the endosperm and/or pericarp which are contributed to a profile of the required carotenoids [24].

The phenotypic color of sorghum endosperm is determined by genotypic factor related to carotenoid biosynthesis [25]. Endosperm usually exhibits yellow or white color. According to the USDA-National Plant Gemplasm System, only 381 accessions of sorghum exhibited yellow endosperm color from a total of 42,869 of total sorghum accessions [24]. When compared with white, yellow endosperm shows higher carotenoid amounts [24]. The total carotenoids contents of selected yellow endosperm sorghum accessions reported by Cardoso were 0.02-0.85 μg/g DW [26]. Fernandez et al. showed that total carotenoid contents in yellow and white endosperm were around 1.68 and 0.20 μg/g DW, respectively [27]. It appeared more carotenoids in yellow endosperm sorghum accessions than that in white had been established [24,27]. When compared with endosperm, the most carotenoids should be presented in the pericarp. However, relatively little data have been published documenting the carotenoid contents in the sorghum pericarp, and virtually nothing is known about the relationship of pericarp pigments with pro-vitamin A status.

The objective of this study was to identify and quantify the carotenoids and pro-vitamin A status in the selected sorghum accessions with various pericarp pigmentations. These innovative results could be significant in identifying particular sorghum varieties that might prevent vitamin A deficiency.

2.3 Materials and methods

2.3.1 Chemicals

Methanol, ethyl acetate, acetone and petroleum ether at HPLC grade were purchased from Thermal Fisher Scientific (Suwanee, GA). Sodium hydroxide was purchased from Thermal

Fisher Scientific (Suwanee, GA), and anhydrous ammonium acetate was purchased from MP Biomedicals, LLC (Solon, OH). Water used in all preparation and analysis was purified through Barnstead E-Pure Deionization System (Dubuque, IA) and filtered using Millipore 0.45 um membrane (Bedford, MA). The internal standard of trans-β-apo-8'-carotenal was obtained from Sigma-Aldrich (St. Louis, MO).

2.3.2 Sample preparation

Nine sorghum accessions were selected on the basis of their endosperm and pericarp colors. Yellow endosperm/brown pericarp (PI 655996), yellow endosperm/yellow pericarp (PI 656096, PI 585374, PI 563448, PI 585351), yellow endosperm/white pericarp (PI 533943, PI560493), and white endosperm/white pericarp (PI 656112, PI565121) were grown in Manhattan, KS during the 2015 summer crop season. All the sorghum was harvested in November 2015, the panicles were threshed, and the seeds were ground to flour by using a Udy Mill. Sorghum flour was placed in centrifuge tubes and wrapped with aluminium-foil paper to minimize carotenoid photooxidantion reaction and immediately stored at -40 ℃. Final carotenoid contents are expressed as nanograms per gram of dry weight flour.

2.3.3 Carotenoid Extractions

The carotenoids were extracted using the methods described by Kean. E [23], Lipkie. T [21] and Kean. E [28] with some modifications. All sample preparations and extractions were performed under amber light to minimize carotenoid degradation. Approximately 2 grams of each dry flour sample dispersed in 8 mL of double-distilled water which containing 0.1% BHT to prevent carotenoid oxidation. The slurry was saponified at 50 ℃ in a water bath for 30 min upon adding

6 mL of 80% NaOH and 2 mL of methanol with vortexing. After saponification, the carotenoids were extracted with 6 mL of petroleum ether and 2 mL of acetone. Samples were vortexed for 30 sec and then centrifuged at 3,500 g for 5 min to insure complete phase separation. The petroleum ether layer was collected and the residue was then re-extracted an additional two times. The petroleum ether fractions were combined and dried under nitrogen gas, re-dissolved in 1,200 μL of 1:1 methanol/ethyl acetate for yellow endosperm/ yellow pericarp sorghum. Other extracts from yellow endosperm/brown pericarp, yellow endosperm/white pericarp, or white endosperm/white pericarp sorghum was re-dissolved in 600 μL of 1:1 methanol/ethyl acetate. Then the dissolved solutions were filtered by a 0.45 μm filter prior to HPLC analysis. A known concentration of the internal standard, i.e., trans-β-apo-8'-carotenal, was added at the beginning of the extraction to account for extraction recovery and quantification equivalence. All the samples were repeated in triplicate.

2.3.4 HPLC analysis

Shimadzu HPLC system (Kyoto, Japan) was used for chromatographic separation. This system was employed by a DGU-20A3 built-in degasser, a LC-20AB solvent delivery pump, a SIL-20ACHT auto-sampler, a CTO-20AC column holding oven, a CBM-20A communicator module, and a SPD-M20A Photodiode Array Detectors. A YMC waters (Milford, MA) C30 reversed phase column (250 mm length, 4.6 mm diameter, 3.0 μm particle size) was used for the carotenoids separations. HPLC separation method was previous reported referred to Kean. E [23,28] with some modification. Elution was performed with mobile phase A methanol/ 1M ammonium acetate (98:2 v/v) and mobile phase B ethyl acetate; gradient expressed as mobile phase B volume was 5-35% for 20 min, and hold 35% for 10 min before returning to 5%. The flow rate

was maintained at 1 mL/min and the column temperature was maintained at 40 ℃. The detector performed a full spectrum scan between 190-800 nm where 450 nm was used for monitoring carotenoids. Trans-β-apo-8'-carotenal was used as an internal standard for adjustment of extraction recovery and quantitation of carotenoid equivalence. Data was analyzed using the LC solution software (Kyoto, Japan).

2.3.5 TOF/MS analysis

Bruker UltraFlexII MALDI-TOF mass spectrometry in Linear negative mode was used to carry out carotenoid identification. The samples were analyzed using 30 mg/mL DHB (2,5-Dihydroxybenzoic acid) matrix solution in ACN (Acetonitrile) and 0.1% TFA (Trifluoroacetic acid).[29] Carotenoid compounds were confirmed by HPLC retention time, monoisotopic mass and absorbance spectra pattern according to previous publication.

2.3.6 Statistical analysis

Data were analyzed using SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA). Results were evaluated by one-way ANOVA. Tukey's post-hoc test was used to assess the differences of individual carotenoids content in various sorghum accessions. The results were presented as means ± SD, and $p < 0.05$ was considered significant.

2.4 Results

2.4.1 Chromatographic separation

The profile of the carotenoid HPLC chromatograms from representative sorghum accessions with various pericarp pigments are shown in **Figure 2.1**. Total five carotenoids

(lutein, zeaxanthin, β-carotene, α-carotene, and β-cryptoxanthin) and internal standard (trans-β-apo8'-carotenal) were eluted within 30 min.

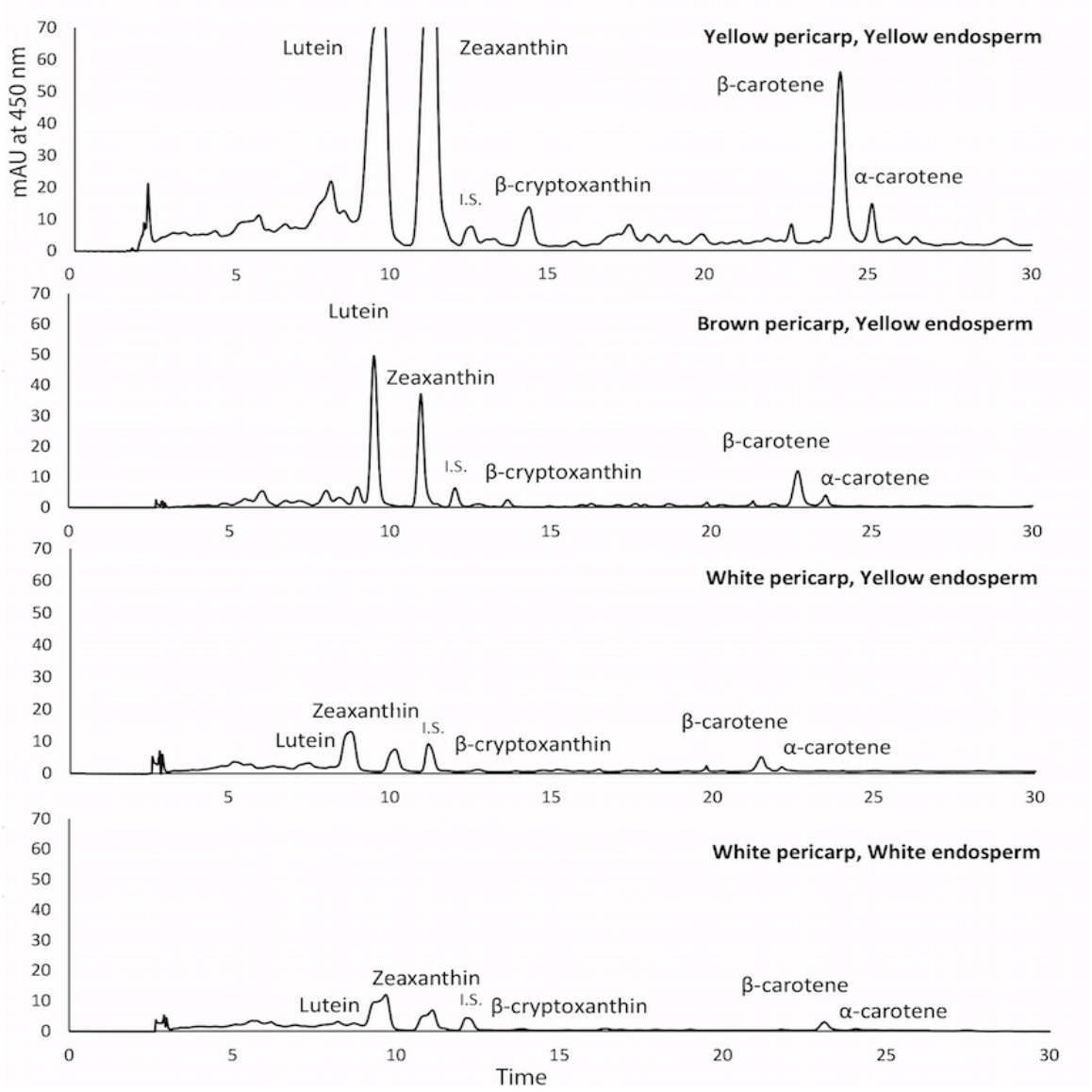

Figure 2.1 Representative HPLC chromatograms of carotenoids detected in the sorghum accessions with various pigments of pericarp and endosperm

2.4.2 Mass spectrometric identification

Following HPLC separation, MALDI-MS data was generated by monitoring the negative ion spectra from 300-1000 m/z. The m/z ratio of each parent mass is listed in **Figure 2.2**. Totally three peaks were identified including lutein and zeaxanthin (m/z 567), α-carotene and β-carotene (m/z 535) and β-cryptoxanthin (m/z 552). As shown in **Figure 2.1**, β-carotene (m/z 535), lutein (m/z 567), and zeaxanthin (m/z 567) are three major carotenoids in sorghum accessions. MALDI-MS did not separate α-carotene from β-carotene or lutein from zeaxanthin, because their molecular weights were identical. However, they could be separated well by HPLC based on their different retention times followed by previous publications [23] **Figure 2.1**.

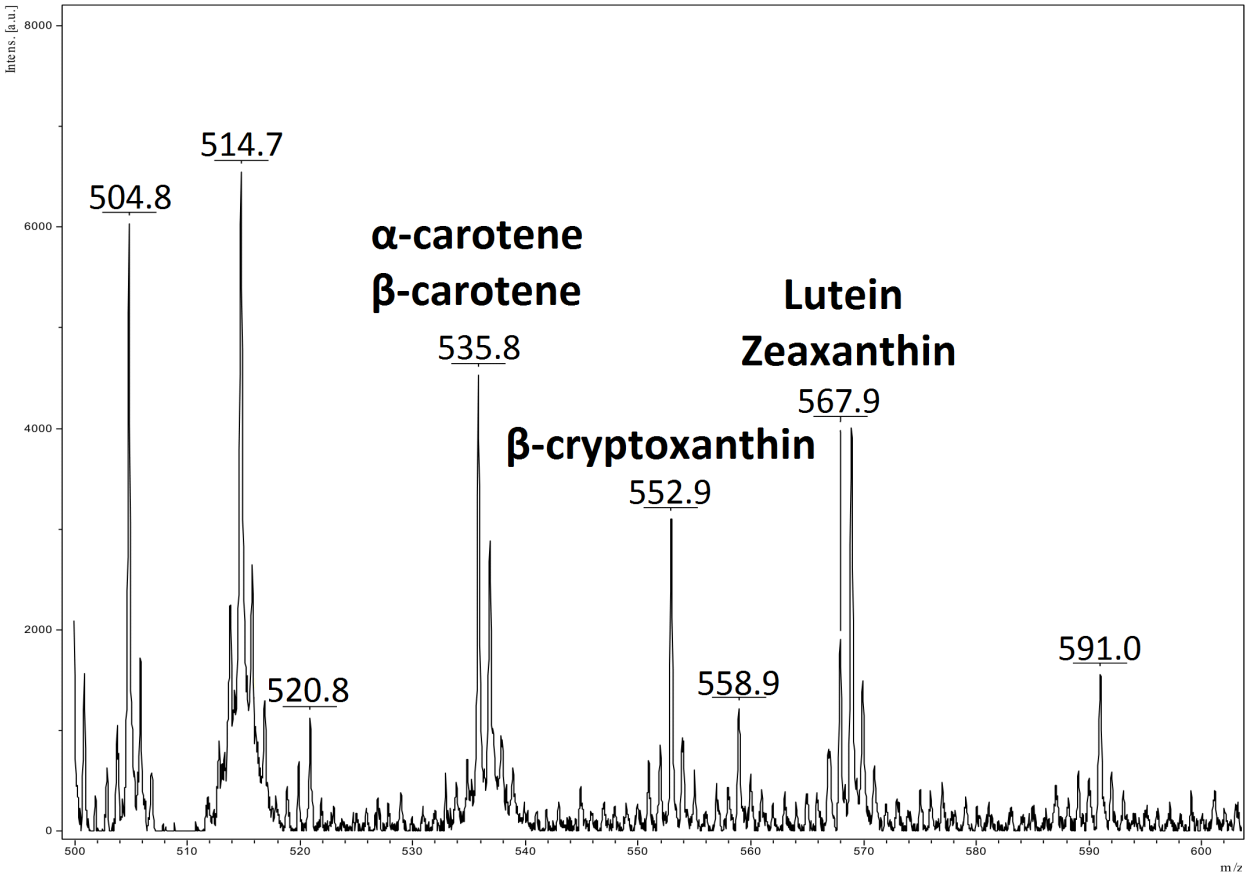

Figure 2.2 Representative TOF-MS of carotenoids identified in the sorghum accessions.

2.4.3 Quantification

The highest contents of total carotenoids were found in sorghum accessions with yellow pericarp/yellow endosperm (PI 656096, PI 585374, PI563448, PI585351), followed by brown pericarp/yellow endosperm (PI 655996). PI 585351 contained a total carotenoid content of 2343.2 ± 96.5 ng/g DW, while PI 655996 was 762.5 ± 103.9 ng/g DW. White pericarp/yellow endosperm (PI 533943, PI 560493) and white pericarp/white endosperm (PI 656112, PI565121) showed the lowest carotenoid contents at 241.2 ± 19.0 and 284.6 ± 25.6 ng/g DW, respectively. As shown in **Figure 2.3**, the contents of individual carotenoid in different pericarp colors demonstrated the similar pattern as the total contents of carotenoids. Top three carotenoids (lutein, zeaxanthin, and β-carotene) accounted for more than half of the total carotenoids. The contents of pro-vitamin A calculated based upon the converted contents of α-carotene, β-carotene, and β-cryptoxanthin were 584.9 ± 38.9 ng/g DW in yellow pericarp/yellow endosperm, 250.6 ± 28.9 ng/g DW in brown pericarp/yellow endosperm, 89.0 ± 12.3 ng/g DW in white pericarp/yellow endosperm, and 79.0 ± 7.3 ng/g DW in white pericarp/white endosperm, respectively. Internal standard trans-β-apo-8'-carotenal was accounted as quantification equivalence and the average recovery is around 60%.

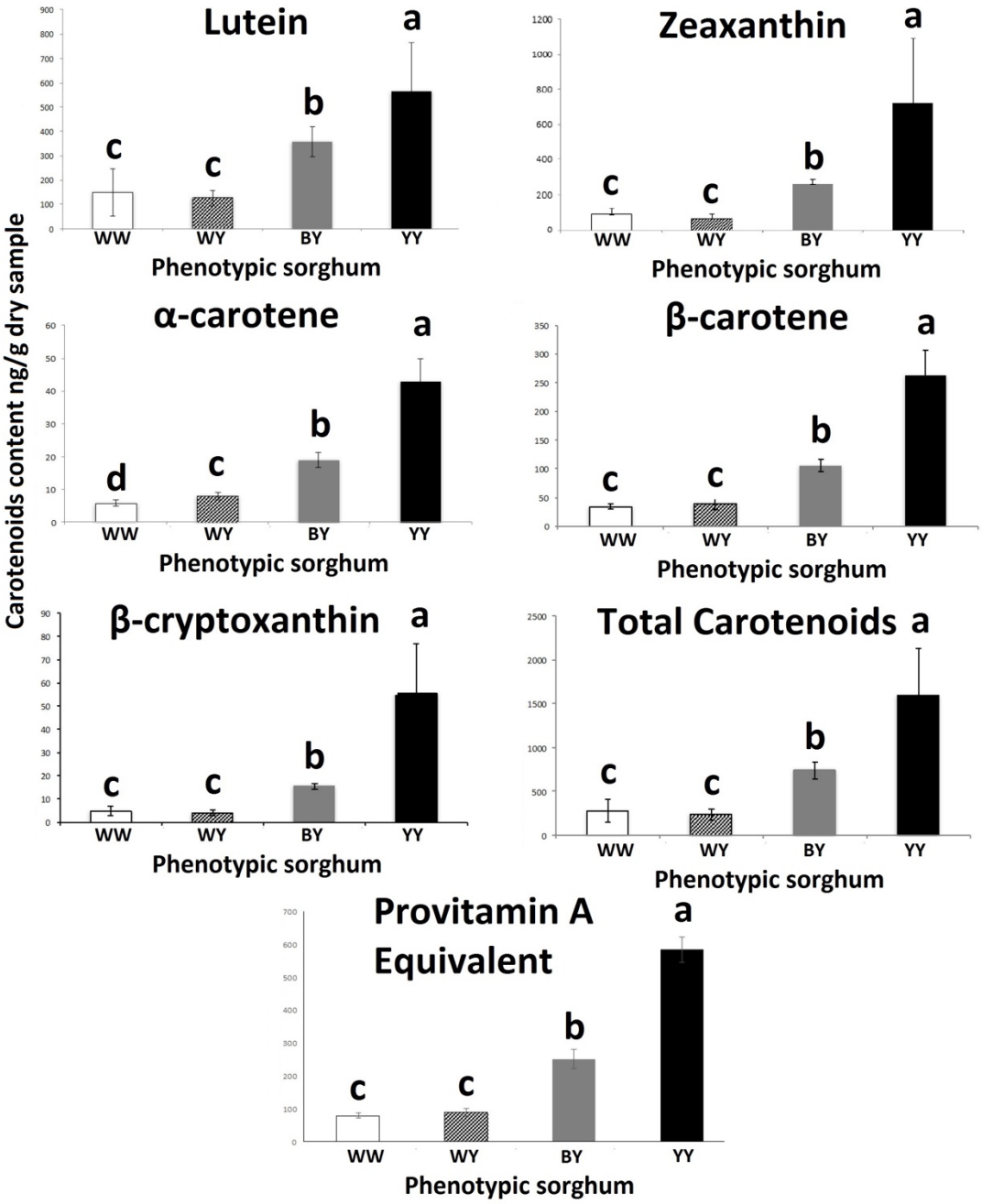

Figure 2.3 The contents of carotenoids and provitamin A in the selected sorghum accessions with various pigments of pericarp and endosperm. YY: yellow pericarp/yellow endosperm; BY: brown pericarp/yellow endosperm; WY: white pericarp/yellow

2.5 Discussion

In total, nine sorghum accessions with different pericarp colors were selected for this pilot study. Five carotenoids including lutein, zeaxanthin, β-carotene, α-carotene, and β-cryptoxanthin were identified and quantitated. Lutein, zeaxanthin and β-carotene were three major carotenoids in sorghum accessions. Yellow pericarp/yellow endosperm sorghum accessions showed the highest contents of carotenoids and vitamin A equivalent, while white pericarp/white endosperm displayed the lowest contents.

The contents of carotenoids detected in this study were higher than the studies reported by others. For example, the contents of lutein and zeaxanthin in the yellow pericarp/yellow endosperm found in this study were much higher than the study reported by Kean et al [23] and Cardoso al [26], but in agreement with Fernandez et al [24]. In addition, the content of β-carotene in yellow endosperm sorghum varieties was also higher than the study reported by Kean et al [23]. Three previous studies mentioned above (Kean et al[23], Cardoso al[26] and Fernandez et al[24]) can only detect two or three carotenoids; however five carotenoids were identified in this study including three pro-vitamin A. A clear reason why the contents of carotenoids detected in this study were higher than the reported studies by others may relate to our improved HPLC method that allowed for a distinct peak separation. In this study, a different C30 column rather than a traditional C18 column was used. When compared with C18, C30 column is much more hydrophobic and thus has better resolution and separation of different geometrical isomers [30–32]. In addition, increase the ethyl acetate concentration could help carotenoid peaks eluted completely within shorter time. There has another 10 minutes to stay with highest concentration of ethyl acetate to enhance the interaction with long-chain molecules and get better peaks. Higher column temperature and less light exposure conducted in this study may also help to

decrease the pressure and oxidative degradation. Although C30 column has been used in Fernandez et al's[24] study, different mobile phase with different gradient of elution may also affect the separation as well as the resolution of peaks. There are many other reasons that may impact the contents of carotenoids such as genetic variation of carotenoid biosynthesis enzymes in sorghum accessions [27] and growth location [27].

Based on the results of this study, five carotenoids identified. Three of them (β-carotene, α-carotene, and β-cryptoxanthin) are precursors of vitamin A. The pro-vitamin A bioactivity is valuable for people in developing countries that may help to improve vitamin A status in their diet. Because of the highest equivalence of pro-vitamin A was found in yellow pericarp and then followed by brown pericarp of this study, there is a trend was seen that the pigments of pericarp might be relevant and highly impact to carotenoid and pro-vitamin A prediction. In addition to endosperm color, pericarp color could be a visual marker for carotenoid contents, this experiment could be thus more significant in helping breeders to select sorghum varieties which contain high pro-vitamin A.

Besides β-carotene, α-carotene, and β-cryptoxanthin have pro-vitamin A activity, lutein and zeaxanthin are the most abundant carotenoids in sorghum grains and they have been reported for some biological function. For example, they are only carotenoids that found in retina[33]. These two antioxidant nutrients may play an important role in the protection against eye disease such as age-related macular degeneration (AMD)[5]. According to NIH National Eye Institute, a nutritional supplement called AREDS could reduce the risk of AMD. The original formulation contains β-carotene, but β-carotene may increase the risk of lung cancer among people whom smoke, so they used lutein and zeaxanthin instead of β-carotene as new formulation AREDS2[34–36]. Lutein and zeaxanthin may be safer and more effective than β-carotene.

This is a pilot study with selected sorghum accessions to provide preliminary data of the contents of carotenoids and pro-vitamin A as well as the association of various pericarp pigments with pro-vitamin A. Future study with more sorghum accessions seems warranted.

In conclusion, total five carotenoids including three pro-vitamin A carotenoids were identified and quantified in the selected sorghum accessions with various pericarp pigments by using LC-MS. The highest contents of total carotenoids and pro-vitamin A were detected in the sorghum accessions with yellow pericarp/yellow endosperm, followed by brown pericarp/yellow endosperm and white pericarp/yellow endosperm. The lowest carotenoids and pro-vitamin A were found in the accessions with white pericarp/white endosperm. It appeared that the phenotypic diversity of sorghum pericarp colors was associated with the content of carotenoids and pro-vitamin A, indicating a different impact of various sorghum varieties on vitamin A deficiency and suggesting a possible prevention of vitamin A deficiency by breeding certain sorghum varieties with pericarp pigments.

2.6 Reference

(1) Maunder, B. *Sorghum: The global grain of the future; National grain sorghum producers:Lubbock, TX*; 2005.

(2) FAO. Food and Agriculture Organization of the United Nations, FAOSTAT database http://faostat.fao.org/.

(3) FALL Ramatoulaye1, CISSE Mady2, SARR Fallou1, Kane Amadou1, D. C. and D. M. Production and Use Sorghum: A Literature Review. *Journal of Nutritional Health & Food Science* 2016, *4* (1), pp 01–04.

(4) C. Anglani. Sorghum for human food- A review. *Plant Foods for Human Nutrition* 1998, *52*, pp 85–95.

(5) Awika, J. M.; Rooney, L. W. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*. 2004, pp 1199–1221.

(6) De Mesa-Stonestreet, N. J.; Alavi, S.; Bean, S. R. Sorghum Proteins: The Concentration, Isolation, Modification, and Food Applications of Kafirins. *Journal of Food Science* 2010, *75*, pp R90–R104.

(7) Duodu, K. .; Taylor, J. R. .; Belton, P. .; Hamaker, B. . Factors affecting sorghum protein digestibility. *Journal of Cereal Science* 2003, *38* (2), pp 117–131.

(8) Food and Agriculture Organization of the United Nations. *Sorghum and millets in human nutrition.*; Food and Agriculture Organization of the United Nations, 1995.

(9) West, K. P. Extent of vitamin A deficiency among preschool children and women of reproductive age. *The Journal of nutrition* 2002, *132* (9 Suppl), pp 2857S – 2866S.

(10) Bendich, A. Biological functions of dietary carotenoids. *Annals of the New York Academy of Sciences* 1993, *691*, pp 61–67.

(11) Paiva, S. a; Russell, R. M. Beta-carotene and other carotenoids as antioxidants. *Journal of the American College of Nutrition* 1999, *18* (5), pp 426–433.

(12) Rao, A. V.; Rao, L. G. Carotenoids and human health. *Pharmacological Research*. 2007, pp 207–216.

(13) Fiedor, J.; Burda, K. Potential role of carotenoids as antioxidants in human health and

disease. *Nutrients* 2014, *6* (2), pp 466–488.

(14) Graham R, R. J. Carotenoids in staple foods: Their potential to improve human nutrition. *Food and Nutrition Bulletin* 2000, *21* (4), pp 404–409.

(15) Tang, G. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *The American journal of clinical nutrition* 2010, *91* (5), pp 1468S – 1473S.

(16) Institute of Medicine, F. and N. B. Beta-carotene and other carotenoids. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. *Washington, D.C.: National Academy Press* 2000, pp 325–400.

(17) Olson, J. A. Recommended dietary intakes (RDI) of vitamin A in humans. *American Journal of Clinical Nutrition* 1987, *45* (4), pp 704–716.

(18) West, K. P. Extent of vitamin A deficiency among preschool children and women of reproductive age. *The Journal of nutrition* 2002, *132* (9 Suppl), pp 2857S – 2866S.

(19) West, K. P.; Darnton-Hill, I. Vitamin A Deficiency. In *Nutrition and Health in Developing Countries*; Humana Press: Totowa, NJ, 2008; pp 377–433.

(20) Adegboye, O. roseline; Smith, C.; Anang, D.; Musa, H. Comparing and Contrasting Three Cultural Food Customs from Nigeria and Analyzing the Nutrient Content of Diets from These Cultures with the Aim of Proffering Nutritional Intervention. *Critical Reviews in Food Science and Nutrition* 2016, *56* (15), pp 2483–2494.

(21) Lipkie, T. E.; De Moura, F. F.; Zhao, Z. Y.; Albertsen, M. C.; Che, P.; Glassman, K.; Ferruzzi, M. G. Bioaccessibility of carotenoids from transgenic provitamin a biofortified sorghum. *Journal of Agricultural and Food Chemistry* 2013, *61* (24), pp 5764–5771.

(22) Kean, E. G.; Bordenave, N.; Ejeta, G.; Hamaker, B. R.; Ferruzzi, M. G. Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. *Journal of Cereal Science* 2011, *54* (3), pp 450–459.

(23) Kean, E. G.; Ejeta, G.; Hamaker, B. R.; Ferruzzi, M. G. Characterization of carotenoid pigments in mature and developing kernels of selected yellow-endosperm sorghum varieties. *Journal of Agricultural and Food Chemistry* 2007, *55* (7), pp 2619–2626.

(24) Fernandez, M. G. S.; Kapran, I.; Souley, S.; Abdou, M.; Maiga, I. H.; Acharya, C. B.;

Hamblin, M. T.; Kresovich, S. Collection and characterization of yellow endosperm sorghums from West Africa for biofortification. *Genetic Resources and Crop Evolution* 2009, *56* (7), pp 991–1000.

(25) Rhodes, D. H.; Hoffmann, L.; Rooney, W. L.; Ramu, P.; Morris, G. P.; Kresovich, S. Genome-wide association study of grain polyphenol concentrations in global sorghum [Sorghum bicolor (L.) Moench] germplasm. *Journal of Agricultural and Food Chemistry* 2014, *62* (45), pp 10916–10927.

(26) Cardoso, L. D. M.; Pinheiro, S. S.; Da Silva, L. L.; De Menezes, C. B.; De Carvalho, C. W. P.; Tardin, F. D.; Queiroz, V. A. V.; Martino, H. S. D.; Pinheiro-Sant'Ana, H. M. Tocochromanols and carotenoids in sorghum (Sorghum bicolor L.): Diversity and stability to the heat treatment. *Food Chemistry* 2015, *172*, pp 900–908.

(27) Salas Fernandez, M. G.; Hamblin, M. T.; Li, L.; Rooney, W. L.; Tuinstra, M. R.; Kresovich, S. Quantitative trait loci analysis of endosperm color and carotenoid content in sorghum grain. *Crop Science* 2008, *48* (5), pp 1732–1743.

(28) Kean, E. G.; Hamaker, B. R.; Ferruzzi, M. G. Carotenoid bioaccessibility from whole grain and degermed maize meal products. *Journal of Agricultural and Food Chemistry* 2008, *56* (21), pp 9918–9926.

(29) Fraser, P. D.; Enfissi, E. M. A.; Goodfellow, M.; Eguchi, T.; Bramley, P. M. Metabolite profiling of plant carotenoids using the matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *The Plant Journal* 2007, *49*, pp 552–564.

(30) Britton, G. Analysis of Carotenoids in Nutritional Studies. In *Carotenoids*; Birkhäuser Basel: Basel, 2009; pp 12–21.

(31) Rivera, S. M.; Canela-Garayoa, R. Analytical tools for the analysis of carotenoids in diverse materials. *Journal of Chromatography A*. 2012, pp 1–10.

(32) Rimmer, C. A.; Sander, L. C.; Wise, S. A. Selectivity of long chain stationary phases in reversed phase liquid chromatography. *Analytical and Bioanalytical Chemistry* 2005, *382* (3), pp 698–707.

(33) Johnson, E. J. The role of carotenoids in human health. *Nutrition in clinical care : an official publication of Tufts University* 2002, *5* (2), pp 56–65.

(34) Clemons, T. E.; Chew, E. Y.; Bressler, S. B.; McBee, W. National Eye Institute Visual Function Questionnaire in the Age-Related Eye Disease Study (AREDS): AREDS Report No. 10. *Archives of ophthalmology* 2003, *121* (2), pp 211–217.

(35) Eye, T. A. AREDS Report No. 8. *Arch Ophthalmol* 2001, *119*, pp 1417–1436.

(36) Age-Related Eye Disease Study Research Group; SanGiovanni, J. P.; Chew, E. Y.; Clemons, T. E.; Ferris, F. L.; Gensler, G.; Lindblad, A. S.; Milton, R. C.; Seddon, J. M.; Sperduto, R. D. The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS Report No. 22. *Archives of ophthalmology (Chicago, Ill. : 1960)* 2007, *125* (9), pp 1225–1232.