Characterization of Carotenoid Pigments in Mature and Developing Kernels of Selected Yellow-Endosperm Sorghum Varieties

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Sorghum is a critical source of food in the semiarid regions of sub-Saharan Africa and India and a potential source of dietary phytochemicals including carotenoids. The objective of this study was to determine the carotenoid profiles of sorghum cultivars, selected on the basis of their yellow-endosperm kernels, at various developmental stages. Following extraction from sorghum flours, carotenoids were separated by high-performance liquid chromatography (HPLC) with diode array detection. Total carotenoid content in fully matured yellow-endosperm sorghum kernels (0.112–0.315 mg/kg) was significantly lower ($p < 0.05$) than that in yellow maize (1.152 mg/kg) at physiological maturity. Variation in total carotenoids and within individual carotenoid species was observed in fully mature sorghum cultivars. For developing kernels, large increases in carotenoid content occurred between 10 and 30 days after half bloom (DAHB), resulting in a peak accumulation between 6.06 and 28.53 μg of total carotenoids per thousand kernels (TK). A significant ($p < 0.05$) decline was noted from 30 to 50 DAHB, resulting in a final carotenoid content of 2.62–15.02 μg/TK total carotenoids. (*all-E*)-Zeaxanthin was the most abundant carotenoid, ranging from 2.22 to 13.29 μg/TK at 30 DAHB. (*all-E*)-β-Carotene was present in modest amounts (0.15–3.83 μg/TK). These data suggest the presence of genetic variation among sorghum cultivars for carotenoid accumulation in developing and mature kernels.

KEYWORDS: Carotenoids; lutein; zeaxanthin; provitamin A; sorghum; maize; HPLC; cereals; development

INTRODUCTION

As indigenous cereal, sorghum [*Sorghum bicolor* (L.) Moench] is a key source of food for both human and animal consumption in many developing nations. Although primarily used as animal feed worldwide, almost 35% of sorghum is grown directly for human consumption in the semiarid regions of sub-Saharan Africa and India (*1*). In these parts of the world, consumption of sorghum can reach as much as 100 kg per capita in a calendar year (*2*). Past studies have focused on the overall nutritional value of the grain, but sorghum may potentially be a unique source of dietary phytochemicals. High molecular weight phenolic compounds such as condensed tannins are commonly associated with sorghum for impairment of digestibility and micronutrients absorption (*3–6*), prompting successful breeding strategies to minimize tannin content. Whereas most research focus has been on tannins, other beneficial phytochemicals including phenolic acids, flavonols, anthocyanins, and, perhaps most interestingly, carotenoids are believed to be found in relative abundance in sorghum (*7–10*).

Carotenoids are a diverse group of yellow-orange pigments found in higher plants, which are broadly divided in two general classes: hydrocarbon carotenes and their hydroxylated derivatives known as xanthophylls (**Figure 1**) (*11*). Several studies have linked diets rich in carotenoids with a reduced risk of several chronic and degenerative diseases including cancer, cardiovascular disorder, and age-related macular degeneration (AMD) (*12–14*). Carotenoid species containing at least 1 unsubstituted β-ionone ring and an attached polyene side chain of 11 carbon atoms in length are potential precursors of retinol (vitamin A). Of the major carotenoid species, α- and β-carotene as well as α- and β-cryptoxanthin are perhaps the most significant dietary sources of vitamin A on a molar basis. This bioactivity is especially valuable in diets of people in developing countries, where vitamin A deficiency is second only to iron deficiency anemia in magnitude as a micronutrient nutritional deficiency (*15, 16*).

Studies on carotenoid content in developing fruits indicate that carotenoid synthesis is increased during the ripening period but that variable levels of final pigmentation, biosynthesis, and
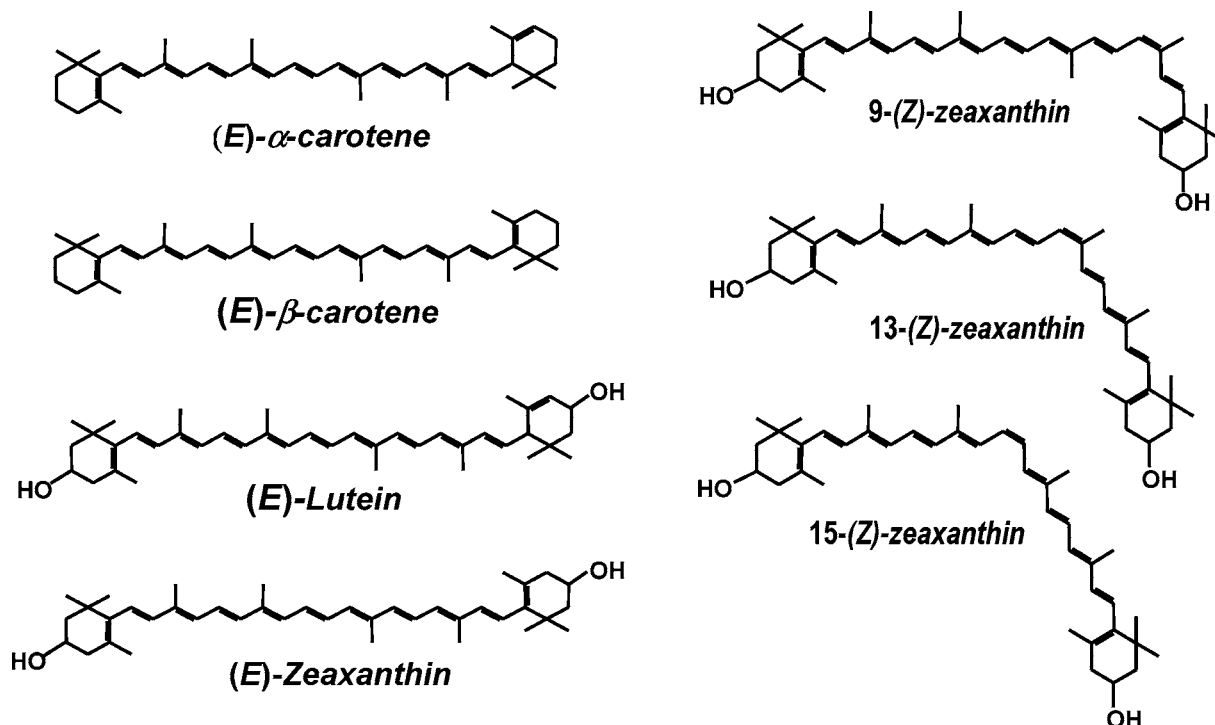
Figure 1. Chemical structures of major carotenoid species found in sorghum flour including zeaxanthin (*Z*)-isomers.

interconversion of carotenoids are maintained among individual fruit species and periods of development (*17*, *18*). Information on grain carotenoid profiles (*19*−*22*) and seed carotenogenesis (*23*−*25*) is more limited and primarily drawn from maize (*Zea mays* L.). Sorghum, as maize, is a member of the grass family Poaceae (*2*). These cereals share several commonalities including similar proximate composition (*26*) and potential accumulation of specific carotenoid pigments (*9*, *10*). Although information is available on maize, knowledge of carotenoid content in sorghum and variations during sorghum kernel development remains extremely limited.

The carotenoid content of select mature yellow-endosperm sorghum varieties was first reported by Blessin et al. (*9*) to reach approximately 1.10−5.60 mg/kg of flour. Although lower than typical yellow maize (*19*−*22*), these data along with efforts by Suryanarayana et al. (*10*) provided initial information on sorghum carotenoid profiles. These early efforts established the ability of sorghum to effectively synthesize and accumulate carotenoids, but provided only limited information on the full carotenoid profile of yellow-endosperm sorghum varieties. A better understanding of the variation of this trait in mature and developing sorghum kernels is required to develop breeding strategies focused on enhancement of beneficial carotenoids.

The present study was undertaken to develop critical knowledge on sorghum carotenoids that will aid in the selection and planning of future breeding strategies to optimize carotenoid profile and content in sorghum crops destined for at-risk populations in developing countries.

MATERIALS AND METHODS

Chemicals and Standards. Extraction and HPLC solvents, acetone, ethyl acetate, methanol, and petroleum ether, were all of certified HPLC and ACS grade. Ammonium acetate (Sigma-Aldrich, St. Louis, MO) was dissolved in double-distilled water and adjusted to pH 4.6 with glacial acetic acid to make a 1.0 mol L^{-1} solution. Analytical standards of lutein, β-carotene (Sigma-Aldrich), zeaxanthin, and β-cryptoxanthin (Indofine Chemical, Hillsborough, NJ) were obtained for HPLC calibration.

Carotenoid Profiling of Mature Yellow-Endosperm Sorghum Varieties. Eight sorghum cultivars were selected on the basis of their yellow-endosperm kernel characteristics and preliminary carotenoid assessment on 2004 sorghum crops (*27*). The breeding lines designated P1181, P1219, P1222, P89006, and P88, three additional white endosperm variants (designated P721, P954063, and P290), and an inbred maize variety, Becks-5538, were selected and grown at the Agronomy Center for Research and Education, near West Lafayette, IN, during the 2005 crop season. All seeds were collected from self-pollinated panicles after physiological maturity (>40 days after pollination), air-dried, and stored at room temperature. Samples used were free from apparent weathering and molding. Thousand kernel weights (TKW) were determined gravimetrically and calculated as mean gram weight of three sets of 1000 kernels expressed on a dry weight basis. The moisture content for each cultivar was determined following American Association of Cereal Chemists (AACC) method 44-15a (*28*). Samples were ground for approximately 5 min at the highest setting on a ball mill (Retsch Vibratory Mill, type MM-2, Brinkmann). Finished flour samples were placed in 50 mL centrifuge tubes, blanketed with nitrogen, sealed, and stored at −80 °C immediately after ground. Carotenoid extraction and analysis were completed within 24 h of grinding. Final carotenoid content is expressed as milligrams per kilogram of flour.

Sorghum Carotenoids Profile during Kernel Development. Four cultivars (P88, P1222, P1181, and P89006) were preselected for further developmental studies based on preliminary screening of the 2004 crop (*27*). These were grown in different unreplicated single-row plots in 2005, at the same location as in the first phase. Several randomly selected plants from each designated cultivar were tagged at 50% anthesis. Three tagged panicles were randomly harvested, during which the top and bottom quarters of the apical and basal sections of the panicles were removed and discarded. Samples were then taken from the remaining middle of the panicles at 10, 20, 30, and 40 days after half bloom (DAHB) and at full maturity (50 DAHB). All samples were placed immediately in polyethylene bags, kept on dry ice during harvesting and transportation, and stored at −80 °C until further treatment.

With the exception of samples for moisture content determination, kernels from all samples were vacuum oven-dried (Lab-line Instruments, Inc., Melrose Park, IL) at 40 °C for 23 h. TKW and moisture content were determined as described above. Samples were milled to flour and

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Table 1. Properties of Matured Sorghum and Maize Cultivars for the Screening Phase of Study[a]

	cultivar								
property	Becks-5538	P88	P1222	P1219	P1181	P89006	P290	P721N	P954063
% moisture[b]	13.34 ± 0.02 a	9.26 ± 0.09 dc	10.82 ± 0.05 b	8.48 ± 0.01 e	9.51 ± 0.05 c	9.65 ± 0.08 c	9.31 ± 0.03 dc	8.83 ± 0.03 de	9.62 ± 0.28 c
TKW[c]	242.15 ± 0.12 a	50.86 ± 0.02 b	35.33 ± 0.01 d	24.73 ± 0.09 i	43.42 ± 0.13 c	34.11 ± 0.20 e	32.91 ± 0.20 f	28.09 ± 0.04 g	27.29 ± 0.08 h
no. of kernels/ 100 g of flour	412 ± 28 i	1966 ± 76 h	2837 ± 44f	4164 ± 17 a	2311 ± 5 g	2938 ± 15 e	3086 ± 19 d	3535 ± 5 c	3690 ± 16 b

[a] Means along rows followed by different letters are significantly different ($p < 0.05$). [b] Moisture percentages are based on triplicate determinations of each cultivar. [c] TKW, thousand kernel weights were determined gravimetrically and calculated as mean (g) weight of three sets of 1000 kernels expressed on a dry weight basis.

stored at −80 °C until extraction and analysis as described above. Carotenoid content is expressed as micrograms per thousand kernels (TK).

Carotenoid Extractions. All sample preparations and extractions were performed under amber light to minimize carotenoid photooxidation and photoisomerization reactions. Approximately ∼1 g of flour was dispersed in 4 mL of double-distilled water. The resulting slurry was then saponified with 30% methanolic NaOH for 30 min in the dark at 37 °C. Following saponification, carotenoids were extracted with 4 mL of petroleum ether/acetone (3:1), containing 0.1% BHT. Samples were vortex mixed for 30 s and then centrifuged at 2500g for 5 min to facilitate phase separation. The petroleum ether layer was collected, and the residue was re-extracted a total of three times. Combined petroleum ether fractions were dried under a stream of nitrogen, resolubilized in 50:50 MeOH/ethyl acetate, and then filtered through a 0.45 μm filter in preparation for LC analysis. Extraction recoveries for lutein, zeaxanthin, and β-carotene, the major carotenoids in sorghum, were determined by spiking a 1 g sample of white-endosperm sorghum variant (P721N) separately with 0.50 μg of the specific carotenoid. The amounts of lutein, zeaxanthin, and β-carotene recovered from extraction of spiked samples ($n = 3$) were determined to be 118.94, 107.42, and 98.71% of the original amounts, respectively.

Instrumentation and Chromatography. Carotenoid analysis was completed with a Hewlett-Packard model 1090A HPLC system equipped with a model diode 79880A diode array detector. Carotenoid separations were achieved using a Grace-Vydac 201TP54 reversed-phase (4.6×250 mm) polymeric C18 column with a guard column containing the same stationary phase (Grace Vydac, Apple Valley, MN). A gradient elution profile based on a binary mobile phase system consisting of methanol/1 M ammonium acetate (98:2 v/v) in phase A and ethyl acetate in phase B was used. A flow rate of 1.0 mL/min was utilized with initial conditions set at 100% A with a linear gradient to 80:20 A/B over 20 min. The gradient was held for 5 min followed by a 5 min linear gradient back to 100% A and equilibration at initial conditions for 5 min for a total analysis time of 30 min. Detection and tentative identification of all carotenoids was accomplished using in-line diode array data between 250 and 600 nm. Quantification of carotenoids was accomplished using multilevel response curves constructed at 450 nm with authentic carotenoid standards at concentration ranges of 0.035−60 μg/mL for lutein, 0.03−40 μg/mL for zeaxanthin, 0.025−8.0 μg/mL for β-cryptoxanthin, and 0.04−6.0 μg/mL for β-carotene. The concentration of each standard was calculated using the specific absorption coefficient ($A^{1\%}$) for each carotenoid (2240 for lutein in ethanol, 2350 for zeaxanthin in ethanol, 2400 for β-cryptoxanthin in hexane, and 2590 for β-carotene in hexane) (29). The observed limits of detection and quantification, functionally defined as 3:1 and 5:1 signal-to-noise, respectively, were determined to be 0.83 and 1.38 ng/25 μL injection of β-carotene. Due to a lack of authentic standards for (Z)-isomers, α-cryptoxanthin, and α-carotene, values were estimated on the basis of the response of closely related carotenoid species. Specifically, lutein + zeaxanthin (Z)-isomers and α-cryptoxanthin were quantified using the standard response curves for zeaxanthin and (E)-lutein, respectively, whereas β-carotene (Z)-isomers and α-carotene were quantified using the response curve of (E)-β-carotene. Intraday coefficients of variation (CV) for extraction and analysis were 2.3, 2.5, 6.1, and 4.1% for (all-E)-lutein, (all-E)-zeaxanthin, (Z)-lutein + (Z)-zeaxanthin, and β-carotene, respectively.

Statistical Analysis. Carotenoid content was expressed as mean ± standard error of the mean (SEM) of three independent observations. Significant differences in carotenoid contents of the various sorghum varieties were determined by analysis of variance (SAS software 9.1; SAS Institute, Cary, NC) with Tukey−Kramer honestly significant difference post hoc test ($\alpha < 0.05$).

RESULTS AND DISCUSSION

HPLC Separation of Sorghum Carotenoids. Resolution of lutein, zeaxanthin, α- and β-cryptoxanthin, and α- and β-carotene was accomplished on a polymeric RP-C18 column within 30 min. Additionally, several (Z)-isomers of lutein, zeaxanthin, and β-carotene were partially resolved from their respective (all-E)-isomers. Tentative identification of lutein and zeaxanthin isomers was aided by comparison of spectral data collected by in-line diode array detection to those previously reported for lutein and zeaxanthin isomers (20, 30). This method compares well to separations reported earlier of lutein and zeaxanthin using C18 column technology (31, 32). However, complete resolution of individual lutein and zeaxanthin (Z)-isomers was hampered by the complexity of the sorghum extracts and limitation of the polymeric C18 column, making identification of individual isomers challenging. Future application of C30 column technology and/or normal phase techniques will be required to provide a more accurate profile of sorghum lutein and zeaxanthin geometrical isomers.

It is important to note that carotenoid (Z)-isomers can be artifacts of extraction and saponification procedures. However, in preliminary experiments assessing the saponification method, formation of specific carotenoid (Z)-isomers was not noted in saponified extracts. In addition, in-line diode array data was utilized to compare potential (Z)-isomers carotenoid absorption spectra with the (all-E). On the basis of similarities in spectra and the presence of a strong absorption between 300 and 350 nm consistent with carotenoid (Z)-isomers (20, 30), it was concluded that these unidentified peaks were most likely (Z)-isomers of lutein and zeaxanthin as well as β-carotene that are present naturally in sorghum grains. The accumulation of measurable levels of carotenoid (Z)-isomers may be a byproduct of extended exposure of sorghum kernels to full sun during maturation. Peaks were therefore tentatively labeled as combined lutein + zeaxanthin (Z)-isomers, providing an adequate estimate of the percentage of total xanthophylls in (Z)-isomer configuration.

Carotenoid Profile of Mature Yellow-Endosperm Sorghum Varieties. General kernel properties of the eight sorghum varieties and yellow maize (Becks-5538) can be seen in **Table 1**. Carotenoid content expressed as milligrams per kilogram from whole grain flour produced from each variety can be seen in **Table 2**. In general, (all-E)-lutein and (all-E)-zeaxanthin were determined to be the predominant carotenoid species in whole grain flours for all cultivars, accounting for >70% of the total

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Table 2. Carotenoid Content in Selected Yellow-Endosperm Sorghum and Maize Flours[a-c]

carotenoid	$t_R{}^d$	Becks-5538	cultivar							
			P88	P1222	P1219	P1181	P89006	P290	P721N	P954063
lutein	6.6	0.511 ± 0.016 a	0.174 ± 0.001 b	0.102 ± 0.003 c	0.078 ± 0.004 c	0.040 ± 0.003 d	0.034 ± 0.001 de	0.009 ± 0.000 e	0.004 ± 0.00 f	0.003 ± 0.000 f
zeaxanthin	7.5	0.286 ± 0.011 a	0.103 ± 0.006 c	0.142 ± 0.006 b	0.115 ± 0.002 c	0.101 ± 0.006 c	0.057 ± 0.003 d	0.007 ± 0.001 e	0.008 ± 0.001 e	0.007 ± 0.000 e
(Z)-L/Z[e]	5.7	0.144 ± 0.004 a	0.020 ± 0.000 c	0.052 ± 0.006 b	0.045 ± 0.001 c	0.016 ± 0.001 c	0.016 ± 0.003 c	ND[f]	ND	ND
α-cryptoxanthin[e]	10.0	0.044 ± 0.003	ND	ND	ND	ND	ND	ND	ND	ND
β-cryptoxanthin	11.3	0.029 ± 0.003	ND	ND	ND	ND	ND	ND	ND	ND
α-carotene[e]	17.2	0.098 ± 0.024	ND	ND	ND	ND	ND	ND	ND	ND
β-carotene	18.1	0.027 ± 0.006 a	0.010 ± 0.001 b	0.007 ± 0.001 b	0.009 ± 0.001 b	0.009 ± 0.001 b	0.005 ± 0.001 b	ND	ND	ND
(Z)-β-carotene[e]	19.9	0.014 ± 0.004 a	0.008 ± 0.001 b	0.005 ± 0.002 b	0.007 ± 0.000 b	0.007 ± 0.000 b	ND	ND	ND	ND
total[b]		1.152 ± 0.023 a	0.315 ± 0.007 b	0.309 ± 0.014 b	0.254 ± 0.006 c	0.173 ± 0.009 d	0.112 ± 0.008 e	0.016 ± 0.001 f	0.012 ± 0.001 f	0.010 ± 0.001 f

[a] Means along rows followed by different letters are significantly different ($p < 0.05$). [b] Total carotenoids and species by dry weight basis and are based on an average of three extractions from each cultivar. [c] All data are expressed as mg of carotenoids per kg of flour. [d] t_R is retention time (min) for C18 separation of carotenoids as described under Materials and Methods. [e] Carotenoid content estimated using response curves for (E)-zeaxanthin [for (Z)-L/Z]; (E)-lutein (for α-cryptoxanthin), and (E)-β-carotene [for (Z)-β-carotene and α-carotene]. [f] ND, not detected.

carotenoid content. (*all-E*)-Lutein (0.511 mg/kg) was the most abundant carotenoid in maize, followed by (*all-E*)-zeaxanthin (0.286 mg/kg), (*Z*)-lutein + zeaxanthin (0.144 mg/kg), α-carotene (∼0.098 mg/kg), α-cryptoxanthin (∼0.044 mg/kg), β-cryptoxanthin (0.029 mg/kg), β-carotene (0.027 mg/kg), and (*Z*)-β-carotene (0.014 mg/kg). These levels are comparable to carotenoid contents reported previously for other maize varieties (*19−21*).

For all sorghum cultivars, total carotenoid content was significantly lower than that for maize, ranging from 0.010 to 0.315 mg/kg (**Table 2**). The three white endosperm varieties (P290, P721N, and P954063) were determined to have only trace levels of total carotenoids (<0.016 mg/kg). Although lower than yellow maize, sorghum cultivars P88, P1222, P1219, and P1181 were found to be significantly higher in total carotenoid content than P89006 (p < 0.05). With the exception of P88, (*all-E*)-zeaxanthin was the most abundant carotenoid in sorghum (0.007−0.142 mg/kg) followed by (*all-E*)-lutein (0.003−0.174 mg/kg), (*Z*)-lutein + zeaxanthin (0.016−0.052 mg/kg), (*all-E*)-β-carotene (0.005−0.010 mg/kg), and (*Z*)-β-carotene (0.005−0.008 mg/kg). α-Cryptoxanthin and β-cryptoxanthin were not detected in sorghum cultivars included in this study.

Whereas yellow maize (Becks-5538) carotenoid content was determined to be almost 5-fold higher over all yellow-endosperm sorghum varieties, interesting qualitative differences were observed. Lutein has been well documented to be the most abundant carotenoid species in many common cereal grains including durum wheat (*33, 34*), barley (*22*), and maize (*23−25, 35, 36*). Published ratios of lutein to zeaxanthin range from 10:1 to ∼2:1 and ∼3:1, respectively, for these grains. Results of our yellow maize analysis are in agreement with these previously published studies. Interestingly, sorghum, with the exception of varieties P88 and P290, maintained a zeaxanthin to lutein ratio of 1.4−2.5, making sorghum similar to several cultivars of white and inbred maize for its seemingly preferential accumulation of zeaxanthin as compared to lutein (*19, 20*). Furthermore, total carotenoid levels in the analyzed sorghum cultivars are similar to those previously published for specific white and inbred maize varieties (*19, 20*). Levels of total lutein and zeaxanthin (*Z*)-isomers (up to 17%) found in sorghum cultivars are similar to the levels in yellow maize (∼13%) analyzed in this study (**Table 2**). Geometric isomers in fresh maize and maize flour have been previously identified (*21, 37, 38*). Interestingly, only low amounts of provitamin A carotenes were found in the sorghum cultivars screened in this study compared to maize Becks-5538, with an average ∼0.14 mg/kg in total carotenes, which was significantly (p < 0.05) higher than all yellow-endosperm sorghum varieties. Combining these data indicates that sorghum is not a particularly rich source of provitamin A carotenoids. However, the presence of appreciable levels of zeaxanthin and lutein is encouraging for sorghum as a source of these bioactive carotenoid pigments.

Sorghum Carotenoid Profiles During Kernel Development. Development of sorghum grains is characterized by the presence of pericarp color change from deep green to yellow. It is believed that these color stages reflect the changes in carotenoid profiles as well as changes in moisture content, kernel size, and density during sorghum development. On the basis of results of a preliminary screening (*27*), four varieties were selected and followed from 10 to 50 DAHB to determine changes in qualitative and quantitative carotenoid profiles as well as key sorghum kernel parameters. For all sorghum varieties TKW increased, whereas percent moisture and number of kernels per 100 g of flour decreased sharply, from 10 to 50

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DAHB (**Table 3**). These results are consistent with cereal grain ontogenesis, when grain moisture typically ceases to increase during the accumulation of storage materials (such as starch) within seeds (*39*).

For all cultivars, total carotenoid content increased from 10 to 30 DAHB, but decreased sharply as the sorghum kernels approached full maturity at 40−50 DAHB (**Figure 2**). At 10 DAHB, variety P1222 was found to have significantly ($p <$ 0.05) higher total carotenoid content (3.83 µg/TK) than cultivars P1181 (2.25 µg/TK), P88 (1.41 µg/TK), and P89006 (1.19 µg/ TK). Large increases in total carotenoid content (∼440−1200%) were found to occur across all varieties from 10 to 30 DAHB, resulting in peak accumulations of 28.53, 19.30, 19.12, and 6.06 µg/TK total carotenoid for varieties P1222, P88, P1181, and P89006, respectively. A significant ($p <$ 0.05) decline was noted from 30 to 50 DAHB, culminating in final carotenoid contents of 15.06, 8.13, 3.09, and 2.62 µg/TK total carotenoid for varieties P1222, P88, P1181, and P89006 respectively. Significant differences among developmental stages were observed between cultivars, with only minor carotenoid accumulation by P89006, whereas P88, P1222, and P1181 showed significant changes over the 50 days of development (**Figure 2**).

Changes in individual carotenoid content mirrored those of total carotenoids during the 50 DAHB developmental study (**Figure 3**). As with the screening study (**Table 2**), (*all-E*)-lutein and-zeaxanthin represented ∼70% of the total carotenoid content for all sorghum varieties assessed. (*all-E*)-Zeaxanthin was the most abundant carotenoid ranging from a low of 2.22 µg/TK in P89006 to a high of 13.29 µg/TK in P1222 at 30 DAHB. (*all-E*)-Lutein was the second most abundant carotenoid from a low of 1.96 µg/TK in PP89006 to a high of 7.18 µg/TK in P1222 at 30 DAHB. (*all-E*)-β-Carotene was present ranging from 0.15 to 3.83 µg/TK. Overall, total β-carotene represented 8.6−18.0% of total carotenoids content among cultivars, with P1222 maintaining the highest total β-carotene level of 3.83 µg/TK at 30 DAHB. Furthermore, as found in the initial screening study (**Table 2**), appreciable levels of total lutein and zeaxanthin (*Z*)-isomers were found in each cultivar at each stage of development (**Figure 3**). Lutein and zeaxanthin present in (*Z*)-isomer form ranged from 5 to 17% of total carotenoid content at all developmental stages (**Figure 3**). Although in only modest amounts, β-carotene (*Z*)-isomers represent a significant portion (18−89%) of the total β-carotene in sorghum after 10− 50 DAHB.

In a similar fashion to total sorghum carotenoid content, the greatest increase in individual carotenoid levels was found to occur between 10 and 30 DAHB. Significant increases were noted for zeaxanthin (366−1873%), β-carotene (383−1065%), (*Z*)-lutein + zeaxanthin (388−970%), and lutein (258−659%), across all varieties. Thousand kernel weights similarly showed an increase over the same period while moisture content decreased (**Table 3**), likely to accommodate more storage materials such as starch. A sharp decline in xanthophylls, zeaxanthin and lutein, was noted in all sorghum varieties on a kernel basis, between 30 and 40 DAHB (**Figure 3**). Variety P1181 demonstrated the most significant loss (from 8.64 to 1.52 µg/TK and from 4.66 to 0.86 µg/TK) in zeaxanthin and lutein, respectively, whereas P89006 showed the least amount of decline (from 2.22 to 1.92 µg/TK and from 1.96 to 1.36 µg/ TK) in zeaxanthin and lutein at 30−40 DAHB, respectively. The apparent loss of these xanthophylls in sorghum is the major factor for the observed drop in total carotenoid content during the same period (**Figure 3**). Reduction in sorghum kernel β-carotene content was more moderate than that of the xantho-

Table 3. Properties of Sorghum Cultivars for the Developmental Phase of Study[a]

DAHB[c]	% moisture				TKW[b]				no. of kernels per 100 g of flour			
	P88	P1222	P89006	P1181	P88	P1222	P89006	P1181	P88	P1222	P89006	P1181
10	75.74 ± 0.08 a	78.37 ± 0.03 a	74.35 ± 0.01 a	76.54 ± 0.06 a	2.83 ± 0.01 e	2.98 ± 0.01 e	2.87 ± 0.01 e	1.79 ± 0.06 e	35341 ± 91 a	33551 ± 117 a	34840 ± 124 a	55818 ± 167 a
20	68.14 ± 0.02 b	67.23 ± 0.04 b	63.22 ± 0.15 b	65.25 ± 0.01 b	11.05 ± 0.01 d	14.04 ± 0.02 d	9.00 ± 0.02 d	11.03 ± 0.08 d	9052 ± 10 b	7132 ± 12 b	11106 ± 22 b	9066 ± 10 b
30	43.39 ± 0.02 c	44.42 ± 0.05 c	42.46 ± 0.58 c	41.37 ± 0.03 c	30.65 ± 0.20 c	25.01 ± 0.05 b	16.93 ± 0.03 c	28.06 ± 0.03 c	3262 ± 21 c	3998 ± 7.45 c	5908 ± 10 c	3563 ± 13 c
40	38.88 ± 0.25 d	35.44 ± 0.19 d	34.34 ± 0.02 d	36.57 ± 0.04 d	39.16 ± 0.20 b	21.76 ± 0.08 c	26.46 ± 0.07 b	30.66 ± 0.04 b	2554 ± 13 d	4596 ± 17 d	3780 ± 11 d	3262 ± 4 c
50	9.85 ± 0.07 e	11.11 ± 0.19 e	9.66 ± 0.02 e	10.44 ± 0.06 e	53.69 ± 0.35 a	29.69 ± 0.38 a	31.50 ± 0.08 a	42.43 ± 0.06 a	1862 ± 12 e	3369 ± 43 e	3174 ± 8 e	2357 ± 10 d

[a] Means within a column followed by different letters are significantly different ($p <$ 0.05). [b] TKW, thousand kernel weights were determined gravimetrically and calculated as mean (g) weight of three sets of 1000 kernels expressed on a dry weight basis. [c] DAHB, days after half-bloom.
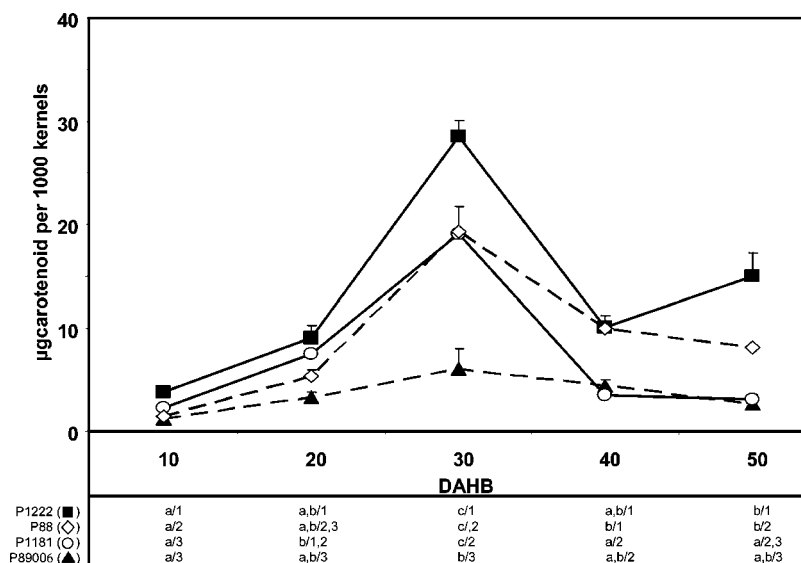
Figure 2. Total carotenoids content of four yellow endosperm sorghum cultivars at 10–50 DAHB: (■) P1222; (◇) P88; (○) P1181; (▲) P89006. Values are expressed as mean ± SEM for three independent observations. The presence of different letters along a row indicates a significant difference in individual carotenoid species content (*p* < 0.05) between sorghum kernels DAHB. The presence of different numbers within a column indicates a significant difference in individual carotenoid species content (*p* < 0.05) within the specified DAHB.
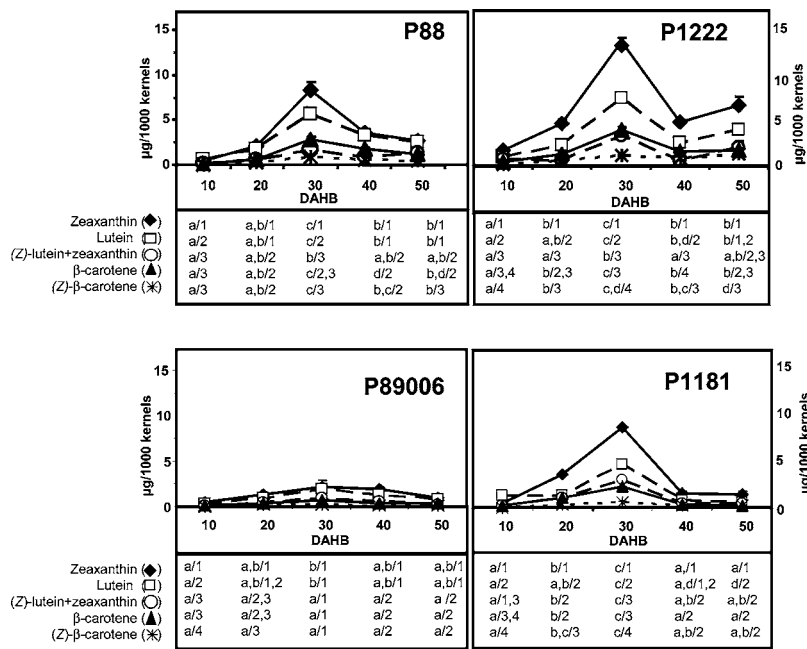

Figure 3. Cultivar P88, P1222, P89006, and P1181 content of (◆) zeaxanthin, (□) lutein, (○) (Z)-lutein + zeaxanthin, (▲) β-carotene, and (∗) (Z)-β-carotene. Values are expressed as mean ± SEM for three independent observations. The presence of different letters along a row indicates a significant difference in individual carotenoid species content (*p* < 0.05) between sorghum kernels DAHB. The presence of different numbers within a column indicates a significant difference in individual carotenoid species content (*p* < 0.05) within the specified DAHB.

phylls. Potential reasons for the sharp and more selective decrease in xanthophyll kernel content may include several factors including the relative abundance of xanthophylls relative to carotenes. Also, sorghum kernels approach full physiological maturity around 40−50 DAHB. It has been reported that in plant seeds, zeaxanthin is an important precursor for abscisic acid, which contributes to seed maturity and dormancy (*40*, *41*). The decline in zeaxanthin content from 30 to 40 DHAB is likely a result of conversion to this vital metabolite as the kernels approach full maturity and prepare for dormancy. Similarly, lutein has been reported to be a precursor for the formation of apocarotenoids such as 3-hydroxy-β-ionone, mycorradicin (with antimicrobial properties), and the generation of several aroma compounds via an oxidative cleavage in cereal plants and in

vitro conditions (*42−45*). As with zeaxanthin, conversion to secondary metabolites likely contributes to the observed reduction in lutein content in sorghum kernels during maturation from 30 to 50 DHAB.

The period from 30 to 50 DAHB seems to be a significant point in sorghum carotenoid synthesis and accumulation. For cultivars P88, P1222, and P1181 (highest in total carotenoids; **Figure 3**), the ratio of β-carotene to zeaxanthin was lower at 30 DHAB compared to physiological maturity. In the carotenoid biosynthesis pathway, hydroxylation of cyclic carotenes is carried out by enzymes specific for β-rings. Conversion of β-carotene to β-cryptoxanthin and eventually zeaxanthin is mediated by these enzyme systems (*45*). It is worth noting that the cultivars with the lowest β-carotene to zeaxanthin ratios

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(P1222 and P1181) showed the highest concentration of zeaxanthin at 30 DAHB. Therefore, it seems that lowered β-carotene content relative to zeaxanthin at 30 DAHB may potentially indicate modulation of carotenoid biosynthetic pathways in the kernels, favoring the increased production of zeaxanthin from β-carotene (**Figure 3**) ultimately required for subsequent abscisic acid synthesis and accumulation. It has been further reported that in cereal grains, although abscisic acid acts prior to full maturity, it is reduced with advanced seed maturation (*40, 41*). Therefore, observed attenuation of zeaxanthin loss between 40 and 50 DAHB (**Figure 3**) may indicate cessation of zeaxanthin conversion to end products such as abscisic acid.

Only minor levels of (*all-E*)- and (*Z*)-β-carotene were detected in sorghum cultivars throughout development (**Figure 3**). This is in agreement with data from initial screening of fully mature sorghum kernels (**Table 2**). Suryanarayana et al. (*10*) previously reported higher β-carotene values in selected yellow-endosperm varieties of sorghum ranging from 0.024 to 0.98 mg/kg of flour. Earlier studies by Blessin et al. (*9*) also reported higher total carotenoid levels in selected yellow-endosperm cultivars, ranging from 1.10 to 5.60 mg/kg of flour. These higher levels of carotenoids are similar to average values of other grains including barley and durum wheat (*22, 33, 34*). Lower levels of total carotenoids and, in particular, β-carotene observed in the present study may be attributed, in part, to factors including varietal difference, specific growth conditions, seed age and maturity, sample storage, growing season, and assessment methodology. Differences in heat and light stresses could also induce major changes in carotenoid profiles as carotenoids are known to be effective photoprotective agents (*47*). Agronomic treatment including the bagging of flowering heads following pollination may serve as a potential source of variation between reported levels. Blessin et al. (*9*) reported that carotenoids content increased by 77% when bagged after pollination compared to standard unprotected open flower heads. Sorghum was not bagged in the present study, potentially resulting in lower carotenoid levels.

Today, sorghum is considered a staple crop and source of food for human consumption in developing countries. The prevalence of vitamin A deficiency in many developing countries has created the need for accessible and acceptable sources of provitamin A carotenoid containing foods. Although varieties of yellow-endosperm sorghum screened in these studies do not appear to be highly concentrated sources of provitamin A carotenoids, they do demonstrate the ability to synthesize and accumulate beneficial oxygenated carotenoids including lutein and zeaxanthin. Current carotenoid levels in yellow-endosperm sorghum varieties screened in this study are not sufficient to reasonably provide the recommended daily allowance of retinol (600−900 retinol activity equivalence per day). However, carotenoid levels of sorghum appear to be similar to those of other grain crops such as oat (*22*) and white maize (*19*) but lower than those of other major grain crops including wheat and yellow maize (*22, 33−37*). The presence of carotenoids, including provitamin A species, in sorghum combined with its wide use as a staple crop in developing countries makes sorghum a primary candidate for ongoing efforts to develop specific lines rich in total and provitamin A carotenoids (*48*). Data from this study provide a conceptual framework from which to develop long-term breeding and genetic strategies to achieve these goals.

To date, carotenoid biosynthesis in cereals such as sorghum has received very little attention. Results from this study demonstrate accumulation of products on both sides: the β,ϵ branch (α-carotene/lutein) and the β,β branch (β-carotene/zeaxanthin) of the biosynthetic pathway. In addition, the higher amount of zeaxanthin relative to lutein found in sorghum cultivars is encouraging both for the development of plant-breeding strategies and as an additional source of dietary zeaxanthin. Furthermore, the presence of significant amounts of β-carotene plant metabolic products such as zeaxanthin indicates the presence of the proper biosynthetic mechanisms for provitamin A β-carotene synthesis and suggests a preference of the β,β branch in sorghum seed carotenogenesis. These data offer evidence of specific targets for plant breeders and/or genetic manipulation to optimize qualitative and quantitative sorghum carotenoid profiles favoring the accumulation of physiologically relevant carotenoid species including provitamin A carotenes as well as lutein and zeaxanthin. Data from the present study, combined with ongoing screening efforts in our laboratory, expand upon earlier reports of sorghum carotenoids and serve as a first step in a broader approach to provide plant breeders critical information facilitating the development of high-carotenoid varieties suitable for human consumption and nutritional intervention.

ABBREVIATIONS USED

RP-HPLC, reversed phase high-performance liquid chromatography; DAHB, days after half-bloom; AMD, age-related macular degeneration; SEM, standard error of the mean; GI, gastrointestinal tract; TKW, thousand kernel weight; AACC, American Association of Cereal Chemists; TK, thousand kernel; tR, retention time.

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