Correlation Between L*a*b* Color Space Values and Carotenoid Content in Pumpkins and Squash (*Cucurbita* spp.)

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Abstract. Carotenoids play an important role in human health by acting as sources of provitamin A or as protective antioxidants. Pumpkins and squash (*Cucurbita* spp.) are excellent dietary sources of carotenoids. The diversity and range of carotenoid types and concentrations within pumpkins and squash provide a means to increase the nutritional value of this crop through plant breeding. Breeding requires reliable estimates of carotenoid types and concentrations to distinguish differences among breeding material. One method used for carotenoid identification and quantification is high-performance liquid chromatography (HPLC). It is a highly sensitive and reproducible method but expensive and time-consuming. In contrast, colorimeters objectively describing visible color are relatively inexpensive and easy to use. The objective of this research was to determine if the carotenoid content within pumpkin and squash measured by HPLC was correlated with colorimeter L*a*b* color space values. Cultigens (cultivars, heirlooms, and PIs) representing white, yellow, and orange flesh color were grown at multiple locations using a randomized complete block design with two replicates at each location. Fruit flesh of each cultigen was evaluated using HPLC and colorimetric analysis. Strong correlations were found between color value a* and total carotenoids ($r = 0.91$) and color value b* and chroma with lutein ($r = 0.87$). Regression equations based on these correlations will be useful for estimating carotenoid type and concentrations. These close associations will also assure that breeding for enhanced carotenoid content within pumpkins and squash can be achieved using an easy-to-use and inexpensive method.

Carotenoids are the principle pigments responsible for the many colors of leaves, fruits, and flowers in plants (Gross, 1991). They act as photoprotective agents and accessory light-harvesting complexes. Carotenoids also play an important role in human health by acting as sources of provitamin A or by acting as protective antioxidants required for proper reproduction, growth, and development; a normal functioning ocular system; epithelial cell integrity; and immune system functionality (FAO/WHO, 2002; Murkovic and Neunteufl, 2002). In vegetables, common provitamin A carotenoids include β -carotene, α -carotene, and β -cryptoxanthin (ODS/NIH, 2006). Other common carotenoids such as lycopene, lutein, and zeaxanthin do not have vitamin A activity but serve as antioxidants.

Pumpkins and squash (*Cucurbita* spp.) are excellent dietary sources of carotenoids (Gross, 1991) and, in 2007, ranked 11th among other vegetables produced around the world (FAO-STAT, 2008). The predominant carotenoids found in pumpkins and squash include lutein, α -carotene, and β -carotene (Gross, 1991). Based on reports by Holden et al. (1999) and Murkovic et al. (2002), the concentrations of lutein, α -carotene, and β -carotene found within *Cucurbita* species and their various fruit types can vary dramatically. In their studies, the fresh weight (FW) range of lutein, α -carotene, and β -carotene in summer-type squash (*C. pepo*) were 0.0 to 21.3 $\mu\text{g}\cdot\text{g}^{-1}$, 0.3 to 1.7 $\mu\text{g}\cdot\text{g}^{-1}$, and 0.6 to 23.0 $\mu\text{g}\cdot\text{g}^{-1}$, respectively. Within winter-type squash (*C. moschata* and *C. maxima*), lutein ranged from 0.8 to 170.0 $\mu\text{g}\cdot\text{g}^{-1}$ FW, α -carotene from 0.0 to 75.0 $\mu\text{g}\cdot\text{g}^{-1}$ FW, and β -carotene from 7.1 to 74.0 $\mu\text{g}\cdot\text{g}^{-1}$ FW.

The flesh colors of pumpkins and squash generally include a wide range of whites, yellows, and oranges (Gross, 1991). This color is based on the particular carotenoid types and concentrations that are influenced by genetic and environmental factors. Over a dozen genes that affect the rind and flesh color of squash have been described (Paris and Nelson Brown, 2005) and include *D*

(dark), *I-1* (light coloration-1), *I-2* (light coloration-2), and *B* (bicolor). Tadmor et al. (2005) studied the effects of these particular genes in different genetic combinations within near-isogenic lines (NILs) of *C. pepo*. In genetic backgrounds that lacked either the dominant *D* and dominant *L-2* alleles, a yellow flesh color developed. In genetic backgrounds with either dominant *D* or *L-2*, a yellow-orange flesh color developed and when the dominant allele of *B* interacts with the dominant allele of *L-2*, an intense orange flesh color will occur. One additional gene that effects squash flesh color is the dominant *Wf* (white flesh), which confers a white flesh color by preventing yellow pigment accumulation (Paris and Nelson Brown, 2005).

The broad range in carotenoid types and concentrations among and within *Cucurbita* species indicates the potential for genetic improvement of these compounds through plant breeding. Accuracy in breeding will require estimates of carotenoid types and their concentrations that are precise enough to distinguish genotypic differences among breeding material. One obstacle is that the extraction and analysis of carotenoid content is time-consuming and expensive. In practical breeding programs, it is not realistic to analyze the carotenoid content of even a small segregating population for selection of genotypes with high levels of carotenoids. An alternative reliable method to estimate carotenoid content and concentration would be beneficial.

High-performance liquid chromatography (HPLC) is used to chemically analyze tissues for carotenoid types and concentrations (Gross, 1991). It is labor-intensive and expensive but a reproducible and highly sensitive process that can separate, identify, purify, and quantify carotenoid levels. In contrast, colorimeters, which objectively measure and describe visible color, are relatively inexpensive and easy to use. The most preferred methods to objectively measure color are the tristimulus Hunter and the CIE L*a*b* systems (Seroczynska et al., 2006). Tristimulus color measurement systems have three color sensors with spectral sensitivity curves similar to that of the human eye and are referred to as color-matching functions (Konica Minolta Photo Imaging, U.S.A., Inc., Mahwah, NJ). These three functions are detected as x, y, and z coordinates, which can be converted into the desired color measurement value system.

Previous studies have correlated color measurement systems with carotenoid content in vegetable crops such as tomato (Arias et al., 2000; D'Souza et al., 1992), sweet-potato (Ameny and Wilson, 1997; Simonne et al., 1993), pepper (Reeves, 1987), and winter-type squash (Francis, 1962; Seroczynska et al., 2006). Interestingly, the authors of these studies differ in their opinions as to how reliable colorimetric analysis would be at estimating carotenoid content and concentrations. If a fair prediction of carotenoid content and concentration could be obtained, this rapid and inexpensive method could be

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very useful in breeding pumpkins and squash for enhanced carotenoid levels. The objective of this research was to determine if the carotenoid content and concentration of pumpkin and squash (*C. moschata* and *C. pepo*) can be correlated with colorimetric analysis using the CIE $L^*a^*b^*$ color value system.

Materials and Methods

Plant material. An initial selection of 15 *C. moschata* and 15 *C. pepo* cultigens (cultivars, heirlooms, and PIs) was made based on subjective descriptions of flesh color with samples representing a range of whites, yellows, and oranges. Cultivars and heirlooms were purchased from commercial seed producers, whereas PIs were obtained from the USDA-ARS North Central (Ames, IA) and Southern (Athens, GA) Regional Plant Introduction Stations.

Field trials. A preliminary field study was conducted 6 Apr. to 15 June 2007 at the Plant Science Research and Education Center (PSREC) located in Citra, FL, to evaluate and select from the 15 *C. moschata* and 15 *C. pepo* cultigens those that would provide a range of white, yellow, and orange flesh color based on colorimetric analysis using the method described subsequently. A randomized complete block design with two replications per cultigen, eight plants per plot, was used. Temperatures recorded by the Florida Automated Weather Network (FAWN) recorded 22 °C average, 38 °C maximum, and 5 °C minimum over the course of the growing season.

From the preliminary study, six *C. moschata* and five *C. pepo* cultigens were selected for both colorimetric and HPLC analysis after plantings at two locations, PSREC and the Gulf Coast Research and Education Center (GCREC), Wimauma, FL, during 11 Mar. to 27 June 2008. Temperatures recorded by FAWN were 22 °C average, 33 °C maximum, and 4 °C minimum and 24 °C average, 38 °C maximum, and 22 °C minimum for PSREC and GCREC, respectively. A randomized complete block design with two replications per cultigen, eight plants per plot, was used at each location. Recommended conventional cultural practices and fertility rates for Florida squash were followed for both 2007 and 2008 (Olson and Simonne, 2007).

Colorimetric and high-performance liquid chromatography analysis. A total of 220 mature fruit (11 cultigens $\times$ five fruit $\times$ two replications per location $\times$ two locations) was harvested for colorimetric and HPLC analysis. Fruit maturity was based on common commercial practices for squash type. Color was recorded using a Minolta CR-400 Colorimeter (Minolta Camera Co., Ltd., Ramsey, NJ) tristimulus color analyzer equipped with an 8-mm diameter measuring area and diffuse illumination of a 2° standard observer. The L^* coordinate indicates darkness or lightness of color and ranges from black (0) to white (100). Coordinates, a^* and b^* ,

indicate color directions: $+a^*$ is the red direction, $-a^*$ is the green direction, $+b^*$ is the yellow direction, and $-b^*$ is the blue direction. Chroma is the saturation or vividness of color. As chromaticity increases, a color becomes more intense; as it decreases, a color becomes more dull. Hue angle is the basic unit of color and can be interpreted, for example, as 0° = red and 90° = yellow. Both chroma and hue are derived from a^* and b^* using the following equations: metric chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$ and metric hue angle: $h = \tan^{-1}(b^*/a^*)$ (degrees).

Slicing each fruit transversely, $L^*a^*b^*$ color space measurements from the edible flesh (mesocarp) of each fruit were recorded within 5 min to avoid discoloration. Our preliminary study revealed replicate measurements of the mesocarp of each fruit per cultigen not significantly ($P \geq 0.05$) different; therefore, avoiding the seed cavity and surrounding tissue (≈ 10 mm), three random measurements per fruit were recorded. A total of 660 (11 cultigens $\times$ five fruit $\times$ three colorimeter measurements per fruit $\times$ two replications per location $\times$ two locations) colorimetric values were recorded.

The separation and quantification of carotenoids were accomplished by HPLC. A total of 22 samples (11 cultigens per location) were prepared for HPLC analysis directly following colorimetric measurements. From each of five fruit harvested per replication, a 10.0 ± 0.1 g FW cubed flesh sample was cut and combined to make a $100.0 \text{ g} \pm 0.5$ g sample per cultigen per location. Samples were vacuum-sealed in plastic storage bags within 50 min of initial fruit slicing, wrapped with aluminum foil, labeled, and held at -20 °C. Samples were then sent frozen to Craft Technologies, Inc. (Wilson, NC) for saponification and HPLC processing. Carotenoids measured included lutein, zeaxanthin, cis-lutein-zeaxanthin, α -carotene, β -carotene, cis- β -carotene, and total carotenoids. All fruit was processed with the colorimeter and prepared for HPLC within 3 d of field harvest and underwent HPLC analysis within 4 weeks of harvest.

Data analysis. Color space values and carotenoid content of the *Cucurbita* cultigens, across locations, were subjected to analysis of variance by the GLM procedure of SAS (Statistical Analysis System Version 9.2; SAS Institute, Cary, NC). Cultigens, locations, replications, fruit, and replicate measurements within fruit were considered as random effects. Least significant differences among cultigens were determined at a 5% significance level. Cultigen means across locations were calculated for each trait. Components of variance were estimated by using the VARCOMP procedure of SAS. Broadsense heritabilities, based on cultigen means, were calculated for each trait according to Fehr (1987). Spearman's coefficient (r_s) of rank correlations (Steel et al., 1997) were calculated to test differences in rank order among the cultigens between the two locations. Pearson correlation coefficients (r) and linear regression (R^2) between color space

values and carotenoid content were calculated from the means of the cultigens across locations using the CORR and REG procedure of SAS. Scatterplots of the data indicated that all relationships between color space values and carotenoid content were linear.

Results and Discussion

Colorimetric evaluation. Significant ($P \leq 0.0001$) differences were observed among cultigens for flesh color represented by the five color space values: L^* , a^* , b^* , chroma, and hue. This demonstrates that genetic variation for flesh color is present among the cultigens tested. Locations were not significantly ($P \geq 0.60$) different for each color space value, but there was a significant ($P < 0.0001$) cultigen-by-location interaction. Based on Spearman's rank correlation tests, no significant ($r_s \geq 0.89$) change in rank order of the cultigens between locations was noted. This indicates that the flesh color of each cultigen was consistent despite different environments. Replicate measurements within fruit of each cultigen were not significantly ($P \geq 0.18$) different, but significant ($P \leq 0.02$) differences were observed for the color space values among fruit within cultigens. Variance component analysis, however, revealed fruit within cultigen estimates to be less than 1.5% of the phenotypic variance for each value. With such a small effect, little would be gained by evaluating more fruit per cultigen. Broadsense heritability (H^2) estimates of the five color space values (Table 1) ranged from 0.81 to 0.93 indicating that genetic improvement and effectiveness of selection for color would be moderate to high.

Subjective observations of flesh color were consistent with the color space value hue in that lower hue angles corresponded to more orange-red flesh and higher hue angles corresponded to more yellow flesh (Table 1). 'Butterbush' (*C. moschata*), with a mean hue angle of 77.5°, represented orange-red flesh color in this study and was significantly ($P \leq 0.05$) different from all other cultigens evaluated. 'PI 314806' (*C. pepo*) reflected a whitish yellow flesh color with a mean hue angle of 102.9°. Differences in hue of the cultigens tested may be the result of the various types and ratios of pigments present.

Mean color space values a^* ($+a^*$ red direction; $-a^*$ green direction) ranged from -4.9 for 'Tennessee Sweet Potato' (*C. moschata*) and 'Fordhook Acorn' (*C. pepo*) to 14.8 for 'Butterbush' (*C. moschata*) (Table 1). The lowest mean color space value, b^* ($+b^*$ yellow direction; $-b^*$ blue direction) was 9.8 found within PI 314806 (*C. pepo*) with the highest, 71.4, found within 'Waltham Butternut' (*C. moschata*). Cultigens with hue angles 90° or greater had yellow flesh, mean a^* values ranging from 0.2 to -4.9 , and mean b^* values ranging from 9.8 to 47.8. Cultigens with hue angles 90° or less had yellow-orange flesh, mean a^* values ranging from 6.6 to 14.8, and mean b^* values ranging from 47.3 to 71.4.

Table 1. Means and broadsense heritability (H^2) estimates of color space values, L^* , a^* , b^* , chroma, and hue^z measured in fruit flesh of 11 *Cucurbita* cultigens.

Cultigen ^y	Species	L^*	a^*	b^*	Chroma	Hue	Color (subjective)
Butterbush	<i>C. moschata</i>	73.7	14.8	65.9	67.7	77.5°	Orange-red
Waltham Butternut	<i>C. moschata</i>	73.4	10.4	71.4	72.2	81.8°	Vivid yellow-orange
Sucrine DuBerry	<i>C. moschata</i>	70.5	6.6	47.3	47.8	82.2°	Dull yellow-orange
Ponca Butternut	<i>C. moschata</i>	74.8	7.3	68.1	68.5	83.9°	Yellow-orange
PI 458728	<i>C. moschata</i>	75.4	0.2	47.8	47.8	90.0°	Dull yellow
Tennessee Sweet Potato	<i>C. moschata</i>	83.2	-4.9	31.5	31.9	99.0°	Light yellow
Table Gold Acorn	<i>C. pepo</i>	76.5	9.4	64.5	65.3	81.9°	Yellow-orange
Table King Bush	<i>C. pepo</i>	83.8	-2.4	43.4	43.5	93.3°	Light yellow
Thelma Sander's Sweet Potato	<i>C. pepo</i>	81.6	-2.5	40.8	40.9	93.8°	Light yellow
Fordhook Acorn	<i>C. pepo</i>	83.4	-4.9	36.3	36.6	97.7°	Light yellow
PI 314806	<i>C. pepo</i>	82.2	-2.2	9.8	10.0	102.9°	Light whitish yellow
Least significant difference (0.05)		0.8	0.8	1.5	1.6	0.8°	
H^2		0.81	0.87	0.93	0.93	0.91	

^zNumeric description of color using $L^*a^*b^*$ CIELAB color space. L^* (lightness or darkness) ranges from black (0) to white (100); a^* color direction in red ($a^* > 0$) or green ($a^* < 0$); b^* color direction in yellow ($b^* > 0$) or blue ($b^* < 0$). Chroma (saturation or vividness) = as chromaticity increases, a color becomes more vivid; as it decreases, a color becomes more dull. Hue (tint of color) = an angular measurement in which 0° = red and 90° = yellow.

^yCultigens include cultivars, heirlooms, and PIs previously selected to represent a range of color from white to orange based on $L^*a^*b^*$ color spaces values.

^xValues shown are based on five fruit $\times$ three colorimetric measurements per fruit $\times$ two replications per location $\times$ two locations per cultigen.

Table 2. Means and broadsense heritability (H^2) estimates of carotenoids ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight) measured in 11 *Cucurbita* cultigens.

Cultigen ^z	Species	Lutein ^y	Zeaxanthin	cis-Lutein/ zeaxanthin	α - carotene	β - carotene	cis- β - carotene	Total carotenoids	Color (subjective)
Butterbush	<i>C. moschata</i>	9.2	0.2	0.9	14.9	15.3	1.7	42.3	Orange-red
Ponca Butternut	<i>C. moschata</i>	8.7	0.1	0.6	1.8	2.1	0.3	13.9	Vivid yellow-orange
Waltham Butternut	<i>C. moschata</i>	17.3	0.1	1.2	3.0	3.8	0.9	26.8	Dull yellow-orange
Sucrine DuBerry	<i>C. moschata</i>	4.3	0.0	0.3	2.6	2.1	0.4	9.7	Yellow-orange
PI 458728	<i>C. moschata</i>	3.9	0.0	0.3	0.4	0.6	0.1	5.3	Dull yellow
Tennessee Sweet Potato	<i>C. moschata</i>	0.6	0.0	0.3	0.0	0.2	0.0	1.1	Light yellow
Table Gold Acorn	<i>C. pepo</i>	9.3	0.2	0.9	0.0	3.6	0.6	14.7	Yellow-orange
Table King Bush	<i>C. pepo</i>	2.6	0.0	0.2	0.0	0.9	0.1	3.9	Light yellow
Thelma Sander's Sweet Potato	<i>C. pepo</i>	1.4	0.0	0.1	0.0	0.6	0.1	2.2	Light yellow
Fordhook Acorn	<i>C. pepo</i>	1.4	0.0	0.1	0.0	0.4	0.0	1.9	Light yellow
PI 314806	<i>C. pepo</i>	0.1	0.0	0.0	0.0	0.0	0.0	0.1	Light whitish yellow
Least significant difference (0.05)		10.4	0.2	0.8	4.1	5.6	0.9	20.4	
H^2		0.37	0.49	0.42	0.85	0.74	0.59	0.59	

^zCultigens include cultivars, heirlooms, and PIs previously selected to represent a range of color from white to orange based on $L^*a^*b^*$ color spaces values.

^ySeparation and quantification of carotenoids were accomplished by high-performance liquid chromatography. Values shown based on 100 g fresh weight samples per cultigen per location.

A range of L^* , lightness (83.8) to darkness (70.5) of color, and chroma, dullness (10.0) to vividness (72.2) of color, was present within the cultigens evaluated (Table 1) and may reflect different concentrations of pigments. Three yellow-orange cultigens with hue values not significantly ($P \geq 0.05$) different from each other included 'Waltham Butternut' (*C. moschata*), 'Sucrine DuBerry' (*C. moschata*), and 'Table Gold Acorn' (*C. pepo*). The flesh color of 'Waltham Butternut' is lighter (mean $L^* = 73.4$) and more vivid (mean chroma = 72.2) compared with 'Sucrine DuBerry's' flesh color, which is darker (mean $L^* = 70.5$) and duller (mean chroma = 47.8). The yellow-orange flesh and rind color of 'Table Gold Acorn' is the result of the introgression of the *B* (*Bicolor*) gene. Typically, acorn-type squash have light yellow flesh color at maturity as reflected by 'Table King Bush' (mean $L^* = 83.8$; mean hue angle = 93.3), 'Thelma Sander's Sweet Potato' (mean $L^* = 81.6$; mean hue angle = 93.8), and 'Fordhook Acorn' (mean $L^* = 83.4$; mean hue angle = 97.7). Several genes that affect rind as well as the flesh color of squash have been described (Paris and Nelson Brown, 2005; Tadmor et al., 2005) including *D* (dark), *I*-1 (light coloration-1), *I*-2 (light coloration-2), and *B* (*bicolor*).

Tadmor et al. (2005) studied the effects of these particular genes in different genetic combinations within NILs of *C. pepo* and reported that when the dominant allele of *B* interacts with the dominant allele of *L*-2, an intense orange rind and flesh color will develop. The flesh color of 'Table Gold Acorn' is lighter (mean $L^* = 76.5$) than both 'Waltham Butternut' and 'Sucrine DuBerry' and falls in between these two cultigens for color vividness (mean chroma = 65.3).

Carotenoid content. Among cultigens tested, significant ($P \leq 0.03$) differences were observed for α -carotene, β -carotene, cis- β -carotene, and total carotenoids suggesting genetic variation in carotenoid accumulation is present. Statistically, no significant differences were observed for lutein ($P = 0.08$), zeaxanthin ($P = 0.07$), or cis-lutein-zeaxanthin ($P = 0.06$), but this may be a reflection of restricted sampling for analysis. In this study, locations were not significantly ($P > 0.05$) different for each carotenoid measured. Broad-sense heritability (H^2) estimates of the carotenoids measured ranged from 0.37 to 0.85 (Table 2) with genetic improvement and effectiveness of selection being most effective for α -carotene ($H^2 = 0.85$) and β -carotene ($H^2 = 0.74$).

On average, orange-red and yellow-orange flesh colored cultigens contained 21.5 $\mu\text{g}\cdot\text{g}^{-1}$ FW total carotenoids, whereas yellow flesh colored cultigens contained 2.4 $\mu\text{g}\cdot\text{g}^{-1}$ FW total carotenoids (Table 2). This represented a ninefold increase in total carotenoids provided within orange-red and yellow-orange flesh-colored cultigens versus yellow flesh-colored cultigens. The most abundant carotenoids within the orange-red and yellow-orange flesh-colored cultigens were lutein (average, 9.8 $\mu\text{g}\cdot\text{g}^{-1}$ FW), α -carotene (average, 4.5 $\mu\text{g}\cdot\text{g}^{-1}$ FW), and β -carotene (average, 5.4 $\mu\text{g}\cdot\text{g}^{-1}$ FW). The most abundant carotenoid within the yellow flesh colored cultigens was lutein (average, 1.7 $\mu\text{g}\cdot\text{g}^{-1}$ FW). Zeaxanthin (range, 0.1–0.2 $\mu\text{g}\cdot\text{g}^{-1}$ FW), cis-lutein-zeaxanthin (range, 0.1–1.2 $\mu\text{g}\cdot\text{g}^{-1}$ FW), and cis- β -carotene (range, 0.1–1.7 $\mu\text{g}\cdot\text{g}^{-1}$ FW) were measured but not found within all cultigens evaluated.

Tadmor et al. (2005) evaluated the carotenoid content of NILs of *C. pepo* differing in fruit pigmentation loci *B* (*bicolor*), *D* (dark), *I*-1 (light coloration-1), and *I*-2 (light coloration-2). In their study, genetic backgrounds that lacked either the dominant *D* or dominant *L*-2 alleles were yellow flesh-colored. On average, these NILs contained 1.2 $\mu\text{g}\cdot\text{g}^{-1}$ FW lutein, 0.6 $\mu\text{g}\cdot\text{g}^{-1}$ FW α -carotene

and β -carotene, and $1.8 \mu\text{g}\cdot\text{g}^{-1}$ FW total carotenoids. Within our yellow flesh-colored *C. pepo* cultigens, similar carotenoid concentrations were obtained with an average lutein concentration of $1.8 \mu\text{g}\cdot\text{g}^{-1}$ FW, an average α -carotene and β -carotene concentration of $0.6 \mu\text{g}\cdot\text{g}^{-1}$ FW, and an average total carotenoid concentration of $2.7 \mu\text{g}\cdot\text{g}^{-1}$ FW (Table 2). In our study, no significant ($P \geq 0.05$) differences among the yellow flesh-colored *C. pepo* cultigens for any of the carotenoids were identified.

In this study, we evaluated 'Table Gold Acorn', a yellow-orange flesh-colored *C. pepo* cultigen. The yellow-orange flesh color of this cultigen is the result of the introgression of the *B* pigmentation allele. In our study, this cultigen had at least a threefold increase in total carotenoid content compared with other *C. pepo* cultigens (Table 2). In the Tadmor et al. (2005) study, the effect of *B* on carotenoid content within their *C. pepo* NILs was dependent on the genetic combinations of pigmentation loci *D*, *I-1*, and *I-2*. In genetic backgrounds with either dominant *D* or dominant *I-1*, the *B/B* genotype had an approximate twofold increase in total carotenoid content. In NILs with *B/B* and *L-2/L-2* genotypes, total carotenoid content increased 10-fold. The genetic makeup of 'Table Gold Acorn' will need to be further investigated to determine its allelic state at the pigmentation loci *D*, *I-1*, and *I-2* and the cause of its increased carotenoid content.

The *C. moschata* cultigen 'Butterbush' contained the highest total carotenoid content ($42.3 \mu\text{g}\cdot\text{g}^{-1}$ FW) and significantly ($P \leq 0.05$) more α -carotene ($14.9 \mu\text{g}\cdot\text{g}^{-1}$ FW) and β -carotene ($15.3 \mu\text{g}\cdot\text{g}^{-1}$ FW) compared with other *C. moschata* cultigens evaluated in this study (Table 2). This suggests genetic variability is present within *C. moschata* that leads to elevated carotenoid content and concentrations. Additional evidence for variability in carotenoid content and concentration within *C. moschata* is provided by Murkovic et al. (2002). The *C. moschata* cultigens in their study averaged $86.7 \mu\text{g}\cdot\text{g}^{-1}$ FW total carotenoids (range, 41.6 – $143.0 \mu\text{g}\cdot\text{g}^{-1}$ FW), $28.2 \mu\text{g}\cdot\text{g}^{-1}$ FW α -carotene (range, 9.8 – $59.0 \mu\text{g}\cdot\text{g}^{-1}$ FW), and $51.0 \mu\text{g}\cdot\text{g}^{-1}$ FW β -carotene (range, 31.0 – $70.0 \mu\text{g}\cdot\text{g}^{-1}$ FW). The carotenoid averages and their ranges were higher than those found in our study. It would be interesting to investigate the genetic basis that leads to elevated carotenoids within *C. moschata* and to compare this with the *C. pepo* findings of Tadmor et al. (2005).

Correlations between color space values and carotenoids. Pearson correlations coefficients (r) between color space values, L^* , a^* , b^* , chroma, and hue, with lutein, α -carotene, β -carotene, and total carotenoids, the four most prominent carotenoid levels measured in this study, were calculated (Table 3). The color value L^* (lightness or darkness) correlated ($P < 0.03$) negatively with lutein ($r = -0.68$) and total carotenoids ($r = -0.66$). A negative correlation between L^* and certain carotenoids would be expected because any increase in pigment

would increase the darkness and thereby decrease L^* . The strength of our two linear relationships, however, would be considered weak ($r \leq -0.70$). The color value a^* (color direction in red or green) was strongly ($r \geq 0.85$) correlated with total carotenoids ($r = 0.91$) followed by moderate ($0.75 \leq r \leq 0.84$) to weak correlations with lutein ($r = 0.84$), β -carotene ($r = 0.77$), and α -carotene ($r = 0.70$). The color value b^* (color direction in yellow or blue) and chroma (saturation or vividness of color) correlated strongly with lutein ($r = 0.87$ for both) and moderately with total carotenoids ($r = 0.75$ and $r = 0.76$). Hue (tint of color, an angular measure) was weakly correlated with α -carotene ($r = -0.62$) and β -carotene ($r = -0.69$) but moderately correlated with lutein ($r = -0.80$) and total carotenoids ($r = -0.83$). The negative correlation observed between hue and the carotenoids measured in this study suggests that as hue angles decrease, carotenoid concentrations would increase.

Studies relating colorimetric values with total carotenoids and/or β -carotene in winter-type squash have previously been reported. Francis (1962) evaluated cultigens of *C. maxima* and *C. moschata* and identified moderate to strong correlations between L^* ($r = -0.78$), a^* ($r = 0.83$), b^* ($r = 0.79$), chroma ($r = 0.93$), and hue ($r = -0.96$) with total carotenoids. In a study evaluating *C. maxima* germplasm, Seroczynska et al. (2006) reported poor to fair correlations between L^* ($r = -0.53$), a^* ($r = 0.77$), b^* ($r = 0.76$), and chroma ($r = 0.77$) with total carotenoids and poor to fair correlations between L^* ($r = -0.54$), a^* ($r = 0.74$), b^* ($r = 0.66$), and chroma ($r = 0.67$) with β -carotene. Comparing our findings with those of Francis (1962) and Seroczynska et al. (2006) revealed similar correlations and strengths, except our correlation between a^* ($r = 0.91$) and total carotenoids was stronger.

In other vegetable crops, studies have reported correlations between colorimetric values and carotenoids. For example, in tomato, both D'Souza et al. (1992) and Arias et al. (2000) found L^* ($r = -0.91$ and $r = -0.92$) and a^* ($r = 0.87$ for both) to be

strongly correlated with lycopene content. In sweetpotato, Simonne et al. (1993) reported a strong positive correlation between hue and β -carotene ($r = 0.99$), whereas Amený and Wilson (1997) reported a moderate correlation between b^* and β -carotene ($r = 0.74$). All four reports viewed colorimetric analysis as an appropriate estimator of carotenoid concentrations. A report by Reeves (1987), however, cautioned readers about making valid assessments of carotenoid concentrations by tristimulus colorimetry. He stated that although correlations may show statistical significance, the variation explained by the correlation may not be adequate enough to be of practical use. In his evaluation of pepper color and carotenoid data, the best parameter to predict total carotenoid content was a negative correlation ($r = -0.713$) with L^* from pureed peppers. In his view, a mathematical equation based on this correlation would, at best, explain only 51% (R^2) of the observed variation between L^* and total pigment content.

Considering the recommendation by Reeves (1987), the strongest linear relationship, in our study, was found between a^* and total carotenoids ($r = 0.91$; $R^2 = 0.83$). The R^2 value indicates that 83% of the variation in total carotenoids can be accounted for by the change in a^* . The color values b^* and chroma were also strongly correlated with lutein ($r = 0.87$; $R^2 = 0.76$ for both). The R^2 values indicate that 76% of the variation in lutein can be accounted for by the change in b^* or chroma. Although regression equations based on these correlations may account for only 76% to 83% of the variation for lutein and total carotenoids, respectively, they may still be useful for estimating these concentrations. Regression equations for the prediction of lutein and total carotenoids based on a^* , b^* , and chroma, based on our findings, are provided in Table 4.

Conclusion

In this study, we found a range of color and carotenoid types and concentrations within pumpkins and squash. This genetic

Table 3. Pearson correlation coefficients (r) between color space values (L^* , a^* , b^* , chroma, and hue) and carotenoids calculated from the means of 11 *Cucurbita* cultigens.

Traits ^a	L^*		a^*		b^*		Chroma		Hue	
	r	P value	r	P value	r	P value	r	P value	r	P value
Lutein	-0.68	0.0209	0.84	0.0013	0.87	0.0006	0.87	0.0006	-0.80	0.0033
α -carotene	-0.49	0.1246	0.70	0.0155	0.45	0.1609	0.47	0.1438	-0.62	0.0433
β -carotene	-0.49	0.1303	0.77	0.0052	0.55	0.0809	0.56	0.0703	-0.69	0.0198
Total carotenoids	-0.66	0.0285	0.91	0.0001	0.75	0.0076	0.76	0.0062	-0.83	0.0017

^aNumeric description of color using $L^*a^*b^*$ CIELAB color space. L^* = lightness or darkness; a^* = color direction in red or green; b^* = color direction in yellow or blue; Chroma = saturation or vividness; Hue = tint of color.

Table 4. Best fit ($r \geq 0.85$) regression equations for pairs of related carotenoids with color space values.

Pairs of relationships ^a	Regression equations	r
Total carotenoids and a^*	Total carotenoids = $6.1226 + 1.7106a^*$	0.91
Lutein and b^*	Lutein = $-6.3881 + 0.2446b^*$	0.87
Lutein and chroma	Lutein = $-6.3743 + 0.2418\text{chroma}$	0.87

^aLutein and total carotenoids in $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight.

variation should make it possible to increase the nutritional value through crossing and selection from within and among the different types with high levels of carotenoids. Based on this study, strong correlations between colorimetric values and carotenoid content were identified. These close associations will assure that indirect selection for high carotenoid content within pumpkin and squash breeding material will be successful, easy to implement, and inexpensive.

Literature Cited

- Ameny, M.A. and P.W. Wilson. 1997. Relationship between Hunter color values and β -carotene contents in white-fleshed African sweetpotatoes (*Ipomoea batatas* Lam). J. Sci. Food Agr. 73:301–306.
- Arias, R., T.C. Lee, L. Logendra, and H. Janes. 2000. Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. J. Agr. Food Chem. 48:1697–1702.
- D'Souza, M.C., S. Singha, and M. Ingle. 1992. Lycopene concentration of tomato fruit can be estimated from chromaticity values. HortScience 27:465–466.
- FAOSTAT. 2008. Crop production stats. 24 Nov. 2008. <<http://faostat.fao.org>>.
- FAO/WHO. 2002. Human vitamin and mineral requirements. Report of a joint Food and Agricultural Organization of the United States (FAO) and World Health Organization (WHO). 24 Nov. 2008. <<http://www.fao.org>>.
- Fehr, W.R. 1987. Heritability, p. 95–105. In: Fehr, W.R. (ed.). Principles of cultivar development, Volume 1, Theory and technique. Macmillian Publishing Company, New York, NY.
- Francis, F.J. 1962. Relationship between flesh color and pigment content in squash. Amer. Soc. Hort. Sci. 81:408–414.
- Gross, J. 1991. Pigments in vegetables: Chlorophylls and carotenoids. VanNostrand Reinhold, New York, NY.
- Holden, J.M., A.L. Eldridge, G.R. Beecher, I.M. Buzzard, S.A. Bhagwat, C.S. Davis, L.W. Douglass, S.E. Gebhardt, D.B. Haytowitz, and S. Schakel. 1999. Carotenoid content of US foods: An update of the database. J. Food Compost. Anal. 12:169–196.
- Murkovic, M.U.M. and H. Neunteufl. 2002. Carotenoid content in different varieties of pumpkins. J. Food Compost. Anal. 15:633–638.
- ODS/NIH. 2006. Dietary supplement fact sheet: Vitamin A and carotenoids. 24 Nov. 2008. <<http://ods.od.nih.gov/factsheets/vitamina.asp>>.
- Olson, S.M. and E. Simonne. 2007. Vegetable production handbook for Florida. University of Florida, IFAS Extension.
- Paris, H.S. and R. Nelson Brown. 2005. The genes of pumpkin and squash. HortScience 40:1620–1630.
- Reeves, M.J. 1987. Re-evaluation of *Capsicum* color data. J. Food Sci. 52:1047–1049.
- Seroczynska, A., A. Korzeniewska, J. Sztangret-Wisniewska, K. Niemirowicz-Szczytt, and M. Gajewski. 2006. Relationship between carotenoid content and flower or fruit flesh colour of winter squash (*Cucurbita maxima* Duch.). Horticulturae 18:51–61.
- Simonne, A.H., S.J. Kays, P.E. Koehler, and R.R. Eitenmiller. 1993. Assessment of beta-carotene content in sweet potato (*Ipomoea batatas* Lam.) breeding lines in relation to dietary requirements. J. Food Compost. Anal. 6:336–345.
- Steel, G.D., J.H. Torrie, and D.A. Dickey. 1997. Principles and procedures of statistics: A biometrical approach. McGraw-Hill, New York, NY.
- Tadmor, Y., H.S. Paris, A. Meir, A.A. Schaffer, and E. Lewinsohn. 2005. Dual role of the pigmentation gene B in affecting carotenoid and vitamin E content in squash (*Cucurbita pepo*) mesocarp. J. Agr. Food Chem. 53:9759–9763.