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Relationship between carotenoids content and flower or fruit flesh colour of winter squash (*Cucurbita maxima* Duch.)

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ABSTRACT

The aim of this study was to find relationship between colour parameters of winter squash flower or fruit flesh and carotenoids content in these parts of a plant. In 2000 – 2004 767 plants of different fruit flesh and flower colour were investigated. Total carotenoids and β-carotene content in flowers and fruits were analyzed and flower and fruit flesh colour were measured in CIE system. For prediction carotenoids and β-carotene content, regression models were calculated, using L*, a*, b* parameters and chroma. Total carotenoids and β-carotene content in flowers showed the strongest correlation with a* parameter and the exponential or linear models were best fitted for this relationship. For total carotenoids or β-carotene content in fruits and fruit flesh colour weaker relationship was found out, and this relationship was stronger for lower values of b* than for higher ones. Multiple linear regression models including L*, a* and b* were also calculated. Flower colour was strongly correlated with carotenoids content in flowers and weakly correlated with carotenoids content in fruits, which suggest that regulation of carotenoids biosynthesis in flowers and fruits is independent.

INTRODUCTION

About 60 different carotenoids occur in plant tissue, including β-carotene, α-carotene, and lycopene. Special physiological activity of these compounds in human organism as the vitamin A precursors and as antioxidants increases interest in looking for methods of determining their content in products (Palace et al. 1999). There is no officially recommended dietary intake of carotenoids, but recommended intake of vitamin A is 1-3 mg per day of retinol equivalent (Murkovic et al. 2002). Pumpkins, especially winter squash cultivars, are considered as a good source of carotenoids. The main carotenoids present in winter squash are α- and β-carotene. Winter squash fruit contains 0.4-7.5 mg 100 g^{-1} of α-carotene and 1.4-8.4 mg 100 g^{-1} of β-carotene, depending on a cultivar (Bushway 1986, Murkovic et al. 2002). According to Holden et al. (1999) the content of β-carotene in cultivars of American origin (in a raw fruit) reaches 4.2 mg 100 g^{-1} and that of α-carotene 0.8 mg 100 g^{-1}. However, USDA reports (Anonymous 2003a, 2003b) show lower value: 0.8 mg 100 g^{-1} of β-carotene and only 0.012 mg 100 g^{-1} of α-carotene. For Polish cultivars 'Amazonka' showed the highest β-carotene content (about 12 mg 100 g^{-1}) (Sztangret et al. 2004). Chemical composition of squash fruits depens on fertilizing method (Paulaskiene et al. 2005). Flowers of cucurbits are also rich in nutritional components and consumed.

Some research was done to find a correlation between colour of plant tissues and carotenoids content. The most preferably used systems of colour measuring of fruit and vegetables are the Hunter tristimulus system and similar CIE L*a*b* system (Lauber et al. 1967, Hunter and Harold 1987, Abbott 1999, Perkins-Veazie and Collins 2004). The relationship between Hunter colour values of edible parts of plants and β-carotene content was examined for sweet potato tubers (Ameny and Wilson 1997). The strongest correlation was found between *L* and *a* values for raw cut tubers (*r* equal to –0.74 and 0.74, respectively). This relationship was investigated also for carrots (Park et al. 1995). Other authors found that a*/b* index for tomato fruits in CIE L*a*b* system showed a highly significant linear regression (R^2 = 96%) with lycopene concentration (Arias et al. 2000). However, weaker correlation (R^2 = 75%) was reported for that relationship in tomatoes in other paper (D'Souza et al. 1992). The light reflectance of apple fruit at 520 nm was strongly correlated with carotenoids content and the index in the form R_{800} ($R_{520}^{-1} - R_{700}^{-1}$) provided a reliable assessment of carotenoids content, ranging from 0.6 to 4.5 nmol cm^{-3} (R^2 = 80%) (Merzlyak et al. 2003).

The aim of present experiment was to find a relationship between flower and fruit flesh colour parameters and carotenoids content in winter squash flower and fruit. Also finding a relationship between flower colour and carotenoids content in fruit was the aim of this study. Such correlation would be useful for selection of high carotene winter squash in breeding programs.

MATERIAL AND METHODS

The experiment was carried out in 2000 – 2004. For measurements and analyses possibly different forms of winter squash were chosen to obtain high variability of carotenoids content in fruits. During period of studies material from total 767 plants was taken to analyses (from cultivars, twelve breeding lines, as well as F_1 and F_2 progeny). Plants were grown in the experimental field of Warsaw Agricultural University. Fruits were harvested at the end of September and then analyzed. Each fruit was cut into small diagonal segments and one part of the segment was used for a chemical analysis and the second part for colour measurement. Before taking colour measurement, diagonal segment of the fruit was cut again and dried with a blotting paper to obtain a flat, smooth, dry surface of the flesh. Each segment was crushed and mixed, then three samples (5 g each) were taken for chemical analysis. Flowers taken for analyses were picked up in July (three fully developed flowers from one plant), and then colour of the petals and carotenoids content were determined.

Samples of fruit tissue (5 g), as well as petals (2.5 g), were ground (4 min, 25 Hz) with 30 g of anhydrous sodium sulfate in the mortar Retsch MM 301 and extracted overnight by 50 ml of hexane. For β-carotene separation the column chromatography method was used (Rutkowska 1981). The column was made up of three layers: anhydrous sodium sulfate (1 cm high), standardized aluminium oxide 90 (5 cm high, decrepitated at the temperature of 104^{o}C per 1 hour) and anhydrous sodium sulfate (1 cm high). β-carotene and total carotenoids content was determined by the spectrophotometer UV-1201V Shimadzu, by using the wavelength 450 nm (Lichtenthaler and Wellburn 1983).

Colour of fruit flesh and flower petals was measured with a HunterLab Miniscan XE spectrophotometer, by using the CIE system (Hunter and Harold 1987), where a value of L* describes lightness (L* = 0 for black, L* = 100 for white), a* describes colour intensity in red (a* > 0) or in green (a* < 0), b* describes colour intensity in yellow (b* > 0) or in blue (b* < 0). The measurements were taken from 8-mm diameter fragments of flesh surface or petals, and the instrument setup was: Illuminant = D65, Observer = 10^o. Before taking measurements, the device was standardized with the light trap and the white tile. Chroma values (C*) were then calculated, according to the formula $C^* = (a^{*2} + b^{*2})^{1/2}$ and hue angle values (H^o) were calculated according to the formula $H^o = \tan^{-1}(b^*/a^*)$. The Universal HunterLab^{TM} software was used to processing colour measurements. For regression analysis of the data Statgraphics Plus v. 4.1 software was applied.

RESULTS AND DISCUSSION

As it can be seen in Table 1, total carotenoids content in analyzed flowers ranged from 1.23 to 18.79 mg 100 g^{-1} f.w. and in fruit flesh from 0.07 to 8.92 mg 100 g^{-1} f.w. In case of β-carotene these values ranged from 1.01 to 13.35 mg 100 g^{-1} f.w., and 0.07 to 6.07 mg 100 g^{-1} f.w., respectively. The values obtained for fruits correspond well with other data reported for *C. maxima* cultivars (Murkovic et al. 2002, Sztangret et al. 2004). However, the highest β-carotene concentration, which was found, exceeded values reported by Holden et al. (1999). Total carotenoids content, as well as β-carotene content in flowers were about two times higher than those in fruit flesh. Also colour measurement showed great differences in CIE values between flowers and fruits (Table 1). L* values for fruits flesh ranged from 56.5 to 85.6, a* from 5.7 to 40.3 and b* from 24.3 to 80.1, but for flowers L* and b* values were much lower and a* values – higher.

Table 1. Differentiation of determined compounds content and CIE parameters values for winter squash flowers and fruit flesh

Compound or parameter	Flowers		Fruits flesh	
	Min. value found	Max. value found	Min. value found	Max. value found
Total carotenoids (mg 100 g^{-1})	1.23	18.79	0.07	8.92
β-carotene (mg 100 g^{-1})	1.01	13.35	0.07	6.07
Dry matter (%)	65.2	78.1	3.0	19.3
L*	1.2	26.1	56.5	85.6
a*	41.9	82.3	5.7	40.3
b*	1.2	18.7	24.3	80.1
C*	42.2	85.7	25.0	86.8

Tables 2 and 3 show results of fitting several regression models to the data obtained for flowers. Since the correlation for hue angle was found much weaker than for other colour parameters, the data for hue angle are not included in the paper. The strongest correlation with total carotenoids or β-carotene content has shown b* parameter and chroma (Table 2 and 3). The exponential model of regression in the form of $Y = (a + bx)^2$ yields the highest correlation value in case of total carotenoids and b* or chroma values. In case of β-carotene content and b* or chroma values linear models yield the highest correlation value (Table 3). Since the *r* coefficient values for the relationships between carotenoids or β-carotene content and colour parameters in CIE system showed so high values (above 0.90), the conclusion can be drown out that there is a very strong and significant ($p < 0.001$) correlation between colour and carotenoids content in winter squash flowers. Fig. 1 shows differentiation between experimentally found values of carotenoids content and theoretically calculated values based on best fitted regression model. As it can be seen, the relationship is very strong and nearly equal for different values of b* and carotenoids contents. Similar differentiation was found for β-carotene content in flowers (data not shown).

Table 2. Relationship between CIE parameters of flowers and total carotenoids content in winter squash flowers

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	$Y = 100.308 – 1.264 L^*$	–0.76	< 0.001
a*	$Y = (1.483 + 0.098 a^*)^2$	0.92	< 0.001
b*	$Y = (–1.464 + 0.066 b^*)^2$	0.95	< 0.001
Chroma C*	$Y = (–1.223 + 0.061 C^*)^2$	0.95	< 0.001

Table 3. Relationship between CIE parameters of flowers and β-carotene content in winter squash flowers

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	Y = 84.509 – 1.074 L*	–0.77	< 0.001
a*	$Y = (1.116 + 0.097 a^*)^2$	0.90	< 0.001
b*	Y = –12.664 + 0.296 b*	0.93	< 0.001
Chroma C*	Y = –11.612 + 0.273 C*	0.92	< 0.001

The corresponding relationships for fruit tissue showed noticeably weaker correlations (Tables 4 and 5). In case of the total carotenoids content, similar and moderately strong relationship with these components a* and b* parameters, as well as chroma, have shown (*r* value about 0.77). The double reciprocal model in the form of $Y = (a + b/x)^{-1}$ yields the highest correlation values. In case of the β-carotene content the highest correlation coefficient was found out for b* parameter and similar (double reciprocal) type of regression function. Since the P-value for total carotenoids and β-carotene contents is less than 0.001, there is a statistically significant relationship between these compounds content and CIE values at the 99.9% confidence level. As it can be seen in Fig. 2, the relationship for carotenoids content is much stronger for low values of a* (up to 25 units), but very weak for high values of this colour parameter. Similar differentiation of the relationship for low and high values of a* we also noted in case of β-carotene content in fruits (data not shown). The reason of this phenomenon is unknown. It seems that other factors besides carotenoids content influence strongly the colour parameters for high values of a*, but these factors are unsignificant for low values of a*.

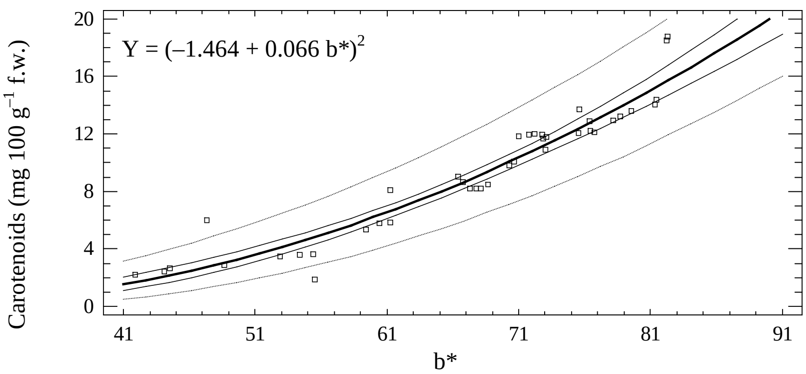

Figure 1. Experimentally found total carotenoids content in winter squash flowers and b* values for the flowers in relation to calculated regression function.

Note: the thick solid line shows the best-fit function, thin solid narrower lines show 95% confidence limits for the carotenoids content for many observations at given values of b*. The broader dotted lines show 95% prediction limits for new observations.

Table 4. Relationship between CIE parameters of fruits flesh and total carotenoids content in winter squash fruits

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	Y = exp(8.579 – 0.12 L*)	–0.53	< 0.001
a*	$Y = (-1.459 + 61.85 / a^*)^{-1}$	0.77	< 0.001
b*	$Y = (-3.623 + 299.64 / b^*)^{-1}$	0.76	< 0.001
Chroma C*	$Y = (-3.323 + 302.39 / C^*)^{-1}$	0.77	< 0.001

Table 5. Relationship between CIE parameters of fruits flesh and β-carotene content in winter squash fruits

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	Y = exp(7.726 – 0.111 L*)	–0.54	< 0.001
a*	Y = exp(–2.316 + 0.090 a*)	0.74	< 0.001
b*	$Y = (-3.251 + 297.98 / b^*)^{-1}$	0.66	< 0.001
Chroma C*	Y = exp(–3.058 + 0.045 C*)	0.67	< 0.001

Other authors reported that a* values show the strongest correlation with the carotenoids content in plant tissues (Park et al. 1995, Sepulveda et al. 1996, Ameny and Wilson 1997, Hyoung 2000). The correlation between a* values and β-carotene content, which was found for the fruits, was similar to this found by Ameny and Wilson (1997) for sweet potatoes (*r* = 0.74) and higher than reported by Park et al. (1995) for carrot (*r* = 0.53). However, cited authors did not describe if these relationships differed for low and high values of a*.

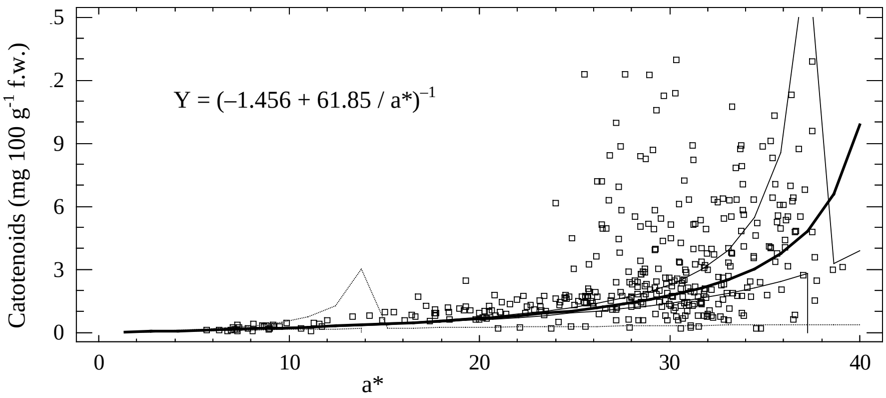

Figure 2. Experimentally found total carotenoids content in winter squash fruits and a* values for the fruits flesh in relation to calculated regression function.
Note: see Fig. 1.

Models of multiple regression were also calculated by using all three basic colour parameters of the CIE L*a*b* system, in order to check if incorporating all these parameters could increase the power of the relationship between colour and carotenoids content. The multiple regression equation for total carotenoids content and flesh colour (*r* = 0.75) is as follows:

$$\text{Log } Y_c = -6.021 + 0.040 \text{ L*} + 0.092 \text{ a*} + 0.021 \text{ b*},$$

and the equation for β-carotene content (*r* = 0.74):

$$\text{Log } Y_\beta = -4.882 + 0.031 \text{ L*} + 0.104 \text{ a*}$$

In these equations: Y_c – total carotenoids content, in mg 100 g^{-1} f.w.; Y_β – β-carotene content, in mg 100 g^{-1} f.w.; L*, a*, b* – CIE parameters.

The b* parameter in the second of equation shown above was eliminated according to procedure due to its low probability level. As it can be seen from these results, the power of the relationship is quite similar to that calculated only for single parameters (a* or b*, respectively).

Results of looking for relationship between flower colour and carotenoids content in fruits are shown in Tables 6 and 7. These results proved that the correlation between flower colour and carotenoids content in fruit is very low. This was found for all CIE colour parameters (*r* value was not greater than 0.30). The relationship between flower colour and carotenoids content in fruit was noticeably lower than that between fruit colour and its chemical composition. This suggests a different regulation mechanism of carotenoids biosynthesis in flower and fruit. Therefore, the colour parameters of flowers cannot be used as credible indicators of total carotenoids or β-carotene content in fruits.

Table 6. Relationship between CIE parameters of flowers and total carotenoids content in winter squash fruits

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	Y = exp(–3.610 + 0.067 L*)	0.18	< 0.001
a*	Y = exp(1.875 – 0.041 a*)	–0.30	< 0.001
b*	$Y = (3.265 - 0.019 \text{ b*})^2$	–0.23	< 0.001
Chroma C*	$Y = (3.240 - 0.018 \text{ C*})^2$	–0.26	< 0.001

Table 7. Relationship between CIE parameters of flowers and β-carotene content in winter squash fruits

Colour parameter	Regression models best fitted	Correlation coefficient *r*	p-value
L*	Y = exp(–2.283 + 0.045 L*)	0.15	0.001
a*	$Y = (1.818 - 0.019 \text{ a*})^2$	–0.23	< 0.001
b*	$Y = (2.182 - 0.0096 \text{ b*})^2$	–0.19	< 0.001
Chroma C*	$Y = (2.170 - 0.009 \text{ C*})^2$	–0.20	< 0.001

CONCLUSIONS

1. Total carotenoids and β-carotene contents in winter squash flowers are very strongly correlated (*r* value above 0.90) with the b* parameter of the flowers.
2. The correlations for fruits are only moderately strong, and a* parameter is best for describing these relationships. For low values of a* the relationships are stronger than for high values of this parameter.
3. Flower colour is strongly correlated with carotenoids content in flowers and weakly correlated with carotenoids content in fruits, so flower colour cannot be used as credible indicator of carotenoids content in fruit.

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ZALEŻNOŚĆ MIĘDZY ZAWARTOŚCIĄ ZWIĄZKÓW KAROTENOIDOWYCH A BARWĄ KWIATÓW I MIĄŻSZU OWOCÓW DYNI OLBRZYMIEJ (*CUCURBITA MAXIMA* DUCH.)

Streszczenie: Celem badań było określenie zależności między parametrami barwy kwiatów i miąższu owoców dyni olbrzymiej a zawartością karotenoidów w tych częściach roślin. W latach 2000 – 2004 przebadano ogółem 767 roślin o zróżnicowanej barwie kwiatów i miąższu owoców. W kwiatach i owocach analizowano zawartość związków karotenoidowych ogółem i β-karotenenu oraz mierzono barwę płatków kwiatów oraz miąższu owoców w systemie CIE. Do określenia zależności między zawartością karotenoidów ogółem i β-karotenu w kwiatach i owocach a ich barwą obliczono modele regresji, wykorzystując parametry barwy L*, a*, b* oraz C*. Zawartość karotenoidów ogółem i β-karotenu w kwiatach wykazywała bardzo silną korelację z wartością parametru a* dla kwiatów. Natomiast zawartość karotenoidów ogółem i β-karotenu w owocach wykazywała umiarkowanie silną korelację z wartością parametru b* miąższu owoców. Zależność ta była większa przy niższych wartościach b*, niż przy wyższych. Obliczono również modele regresji wielokrotnej, uwzględniając wartości L*, a* i b*. Barwa kwiatów była silnie skorelowana z zawartością związków karotenoidowych w płatkach kwiatowych oraz słabo skorelowana z ich zawartością w owocach, co sugeruje, że regulacja biosyntezy związków karotenoidowych w kwiatach i owocach dyni jest niezależna.

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