

Carotenoid Accumulation by Carrot Storage Roots in Relation to Nitrogen Fertilization Level

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Abstract

The accumulation of carotenoid compounds in carrot (*Daucus carota* L.) roots of five cultivars, in relation to different nitrogen fertilization levels was investigated. The experiment was carried out at the Warsaw University of Life Sciences. Carrot cultivars 'Karatani' F₁, 'Trafford' F₁, 'Krakow' F₁, 'Komarno' F₁ and 'HY 7842' were used in the study. Nitrogen fertilization was applied as urea form, in doses ranging from 0 to 120 kg N ha⁻¹, and on two terms: pre-sowing and in the middle of the growing season. The carrot seeds were sown at the beginning of May and the roots were harvested in mid-October at maturity. Total carotenoids and β-carotene contents in the carrot roots were determined after the harvest by means of the standard spectrophotometric method. CIE L*a*b* colour parameters of the roots and the juice, as well as dry matter in the roots were determined. Results of the experiment showed that carotenoid accumulation in the roots was significantly affected by carrot genotype. 'HY7842' carrot showed the highest accumulation of total carotenoids and β-carotene. Nitrogen in the rates applied, did not significantly influence carotenoid content in the roots. Moderate correlation between carotenoid content and colour a* parameter (redness intensity) of the roots and the juice was found.

Keywords: carotenoids, β-carotene, carrot, colour, fertilization, nitrogen

Introduction

Carrot (*Daucus carota* L.) storage roots are widely used for industrial processing, to produce juice and other food products. Carrots grown for food industry especially those used in baby food industry should meet strict quality requirements. High carotenoids and low nitrates content are the most important quality traits of carrot roots (Chen *et al.*, 1995). To achieve low nitrates content in carrots, fertilization with the lowest possible nitrogen rate is recommended. The recommended nitrogen in soil for carrots cultivation ranges from 100 to 140 mg dm⁻³ (N-NO₃ + N-NH₄) and recommended rates of nitrogen in fertilization ranges from 80 to 140 kg ha⁻¹ (Sady *et al.*, 2000; Smoleń and Sady, 2009).

Carotenoid compounds are isoprenoid plant pigments of yellow, orange or red colour (Sandmann, 2001). They show high antioxidant activity and are converted in the human body into vitamin A (Handelman, 2001). Experimental studies suggest that a high consumption of vegetables and fruits rich in carotenoid protects the body against certain kinds of cancer, cataract, cardiovascular diseases and other disorders resulting from free radical activity (Kopsell and Kopsell, 2006). The main carotenoid compounds in carrots are α- and β-carotene (Simon and Wolff, 1987; Surlis *et al.*, 2004). According to Holden

et al. (1999), raw carrot roots contain, on average, 5.7 mg 100 g⁻¹ of β-carotene. Similar values are reported in USDA database (Anonymous, 2004). However, genetic factors and environmental conditions largely influence carotenoids content in carrot roots (Lee 1986; Sandhu *et al.* 1988; Warman and Havard, 1997; Seljasen *et al.*, 2001; Kidmose *et al.*, 2004; Brunsgaard *et al.*, 2006; Gajewski and Dąbrowska, 2007). It was reported that the higher the age of the root, the higher the carotenoid content in carrot roots, so the term harvest is of great importance in practice (Rosenfeld, 1998). Carrot breeders work worldwide to obtain carrot genotypes containing more carotenoids in the root tissue. According to Alasalvar *et al.* (2001), purple-root carrots are especially rich in β-carotene and contain more than 2.2 times more of this compound compared to orange-coloured types. Skrede *et al.* (1997) reported that carrot roots of high carotenoid content showed more reddish and darker colour, but less intensive hue. The relationship between carotenoid content and carrot root colour is yet to be determined.

The objective of the study was to determine carotenoid compounds accumulated in storage roots of five different carrot cultivars commonly used for industrial processing, in relation to the differentiated nitrogen fertilization level. Also the relationship between carotenoid content in

carrot roots and colour parameters of the roots and juice made from the roots was studied.

Materials and methods

The study was carried out at the Warsaw University of Life Sciences. Carrots were cultivated in the 2007-2008 growing seasons on a podzolic soil, of 1.6-1.8% humus content. The experiment was established as a two-factor one, in the split-plot scheme, in four replicates. The plot area in one replicate was 20 m². The factors for the experiment were:

-factor A: nitrogen rates (as below). Nitrogen was applied in the form of urea, two times, first at pre-sowing, at the beginning of May and second at middle-season in the middle of July using the following nitrogen rates (pure N form):

a₁ - 120 kg N ha⁻¹, pre-sowing (total 120 kg)

a₂ - 60 kg N ha⁻¹, pre-sowing (total 60 kg)

a₃ - 30 kg N ha⁻¹ pre-sowing + 30 kg N ha⁻¹ middle-season (total 60 kg)

a₄ - 60 kg N ha⁻¹, middle-season (total 60 kg)

a₅ - control, no N fertilization

-factor B: genotype: carrot cultivars 'Karotan' F₁, 'Trafford' F₁, 'Krakow' F₁, 'Komarno' F₁, 'HY7842'.

The above-mentioned hybrid carrot cultivars are commonly used for industrial processing, to produce juice and concentrate. The 'HY 7842' is an experimental hybrid cultivar, bred by Rijk Zwaan, Holland, especially for industrial processing.

Soil analysis, performed in spring, before N fertilization, showed that the basic nitrogen content (in the form of NO₃) was equal to 30-35 mg dm⁻³ of soil. Therefore, nitrogen amounts in the soil achieved after N fertilization, according to doses shown above, were 90-95, 60-65 and 30-35 mg dm⁻³ of soil, respectively. Besides nitrogen, the following non-nitrogen nutrients were applied before sowing: 170 kg of K₂O (in the form of potassium sulphate), 60 kg of P₂O₅ (in the form of Superfosfat fertilizer) and 30 kg ha⁻¹ of MgO (in the form of magnesium sulphate). In both years carrot seeds were sown at the beginning of May at the rate of 800,000 seeds per ha. The carrot plants were cultivated on raised beds of 30 cm height. The carrots were harvested in mid-October, when the roots were fully mature, and subjected to analysis and measurements.

Total carotenoids and β-carotene contents in the roots were determined with the standard spectrophotometric method (PN-90/A-75101.12). Deeply frozen samples of the roots (5 g) were grounded (4 min, 25 Hz) with 30 g of anhydrous sodium sulfate in a mortar and extracted overnight with 50 ml hexane. For the β-carotene separation column the chromatographic method was used. The column was made up of three layers: anhydrous sodium sulfate (1 cm high), standardized aluminum oxide 90 (5 cm high, decrepitated at the temperature of 104°C per 1 h) and anhydrous sodium sulfate (1 cm high). Carote-

noids content was determined with the UV-1201V Shimadzu spectrophotometer (Shimadzu, Japan), using the wavelength of 450 nm.

Carrot juice was obtained from fresh roots in a professional, rotary juice-maker (Bosch, Germany). Colour parameters of carrot roots and juice were determined with HunterLab XE spectrophotometer (Hunter Lab, USA), using CIE L*a*b* scale, with 10°/D65 setup. The raw data was processed by "Universe" software. Dry matter of the roots was determined by drying samples at the temperature of 105°C until stable weight. All measurements were done on representative root samples, in three replicates.

For the two-way analysis of variance "Statgraphics Plus 4.1" software was applied, and Tukey's HSD (honesty significance test) test was used to show which means differ significantly at probability level of P=0.05.

Results and discussion

It is known that carrot roots are a good source of carotenoid compounds for human, so factors influencing carotenoid accumulation in the roots are of great practical importance. On the other hand, there is a need to decrease nitrogen fertilization in vegetable production practice to obtain safer, low-nitrate food. Carrots show strong tendency to accumulate nitrates in their tissues (Brunsgaard *et al.*, 2006). Restricted nitrogen fertilization, enabled to keep nitrate concentration in the roots to safe levels is one of the recommended countermeasures. However, the possibility of negative effects of restricted nitrogen applications on the carrot yield and quality traits should be considered. In our experiment we applied nitrogen in the form of urea, which is the recommended form for carrots cultivation (Sady *et al.*, 2000). It was found that the effect of different doses of nitrogen fertilization had insignificant effect on carotenoid accumulation in carrot roots (Tab. 1). The results are in agreement with Smoleń and Sady (2009), who reported an insignificant relationship between the nitrogen fertilization level, that ranged from 70 to 140 kg ha⁻¹ and carotenoid accumulation in carrot roots. How-

Tab. 1. Total carotenoids content in carrot roots, in relation to N fertilization and cultivars (mg 100 g⁻¹)

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	8.80	8.50	5.20	8.60	10.75	8.37 ^a
60 + 0	8.95	7.80	7.75	9.55	8.10	8.43 ^a
30 + 30	11.25	7.90	8.00	10.15	9.85	9.43 ^a
0 + 60	7.90	7.00	5.90	7.60	9.70	7.62 ^a
0	7.10	6.50	7.80	7.70	9.95	7.81 ^a
Means for cultivars	8.80 ^b	7.54 ^a	6.93 ^a	8.72 ^b	9.67 ^c	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

Tab. 2. β -carotene content in carrot roots, in relation to N fertilization and cultivars (mg 100 g⁻¹)

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	3.60	3.45	2.20	3.55	4.50	3.46 ^a
60 + 0	3.50	3.10	3.15	3.80	3.35	3.38 ^a
30 + 30	4.55	3.25	3.30	4.00	3.95	3.81 ^a
0 + 60	3.20	2.85	2.35	3.10	3.95	3.09 ^a
0	2.80	2.50	3.05	3.00	3.90	3.05 ^a
Means for cultivars	3.53 ^b	3.03 ^a	2.81 ^a	3.49 ^b	3.93 ^c	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

ever, the results showed significant effects of genotype on carotenoid accumulation in the roots. The highest carotenoid content was recorded in 'HY 7842' roots (6.9 mg 100 g⁻¹, on average), and the lowest in 'Krakow' roots (9.7 mg 100 g⁻¹). This is in accordance with the carotenoid content reported by Simon and Wolff (1987) and Holden *et al.* (1999). The results of the present study show that β -carotene content in carrot roots accounted for about 30-40% of the total carotenoid content (Tab. 2). There was also no evident relationship between β -carotene accumulation in the roots and nitrogen fertilization levels.

The colour of carrot roots results from pigment accu-

Tab. 4. Colour parameter a* of carrot roots, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	34.7	35.1	32.7	33.8	39.2	38.4 ^a
60 + 0	35.9	36.0	31.3	35.1	36.5	37.2 ^a
30 + 30	33.0	34.2	31.5	35.2	38.7	38.9 ^a
0 + 60	34.4	36.6	31.7	34.8	41.3	35.6 ^a
0	36.5	35.7	33.7	32.2	38.6	38.5 ^a
Means for cultivars	34.9 ^b	35.5 ^b	32.2 ^a	34.2 ^b	38.8 ^c	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

mulation in the root tissue. The roots of the investigated carrot cultivars differed with respect to L*, a* and b* colour parameters. The highest L* value, related to the lightness, was found for roots of 'Karotan' and 'Trafford' carrots (Tab. 3). In the case of a* and b* parameters, related to the redness and yellowness, respectively, the highest values were observed for 'HY7842' carrots (Tab. 4 and Tab. 5). The L* and a* colour parameters of the roots were not significantly related to nitrogen fertilization. However, b* parameter (yellowness) of the roots showed the highest value when the dose of 60 kg ha⁻¹ of nitrogen was applied in mid-season.

Tab. 3. Colour parameter L* of carrot roots, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	60.1	61.9	57.2	57.5	59.1	59.1 ^a
60 + 0	59.1	59.8	60.8	56.9	55.2	58.3 ^a
30 + 30	56.8	60.2	61.1	55.8	56.5	58.1 ^a
0 + 60	60.1	58.4	54.6	58.8	56.8	57.7 ^a
0	63.4	60.3	57.9	52.6	58.8	58.6 ^a
Means for cultivars	59.9 ^{cd}	60.1 ^d	58.3 ^{bc}	56.3 ^a	57.3 ^{ab}	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

The colour of carrot juice is especially important when carrots are grown for industrial use. There were no significant differences with respect to the L* parameter of the juice, obtained from the five carrot genotypes (Tab. 6), but significant differences were recorded in a* and b* parameters of the juice. The highest a* value was characteristic for the juice of 'Krakow' (Tab. 7), and the highest b* value for the juice of 'Trafford' and 'Krakow' carrots (Tab. 8). The influence of nitrogen fertilization on colour parameters of the carrot juice was found in the case of a* parameter only, and the highest value of this parameter was observed for carrots which were fertilized with the 60 kg N ha⁻¹ pre-

Tab. 5. Colour parameter b* of carrot roots, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	42.7	47.3	42.9	44.0	49.2	45.7 ^a
60 + 0	44.7	46.7	44.6	46.6	47.1	43.9 ^a
30 + 30	39.9	47.1	42.4	44.0	48.7	45.9 ^a
0 + 60	42.1	48.3	43.6	45.8	49.7	49.0 ^b
0	45.3	48.1	46.1	41.6	48.4	46.4 ^a
Means for cultivars	42.9 ^a	47.5 ^b	43.9 ^a	44.4 ^a	48.6 ^b	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

sowing, and also with the divided dose of 60 kg N ha⁻¹ (Tab. 7).

Dry matter content in carrot roots, fertilized with different nitrogen doses, ranged from 10.9 to 12.1% (Tab. 9). 'Trafford' and 'Karotan' showed the highest dry matter content. For the 'Trafford' carrot cultivar, the dry matter content exceeded even the 13% level. The influence of nitrogen fertilization on the dry matter content in the roots was unclear, since no relationship was found.

In this study it has been tried to determine a relationship between carotenoids content in the carrot roots and CIE colour parameters of the roots and the juice (Tab.

Tab. 6. Colour parameter L* of carrot juice, in relation to N fertilization rates and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	43.8	46.6	46.5	44.4	44.1	45.2 ^a
60 + 0	44.3	45.0	44.5	44.3	44.8	45.9 ^a
30 + 30	43.3	44.8	46.2	43.9	44.6	46.1 ^a
0 + 60	44.9	45.7	45.9	44.2	45.2	45.8 ^a
0	45.1	45.2	45.9	44.2	44.7	45.3 ^a
Means for cultivars	44.3 ^a	45.5 ^a	45.8 ^a	44.2 ^a	44.7 ^a	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

10). The correlation between carotenoids and colour parameters was very weak except those between carotenoids content in the roots and a* parameter of the roots and juice, which were moderately strong. . Therefore, it seems that carotenoids accumulated in carrot roots exert more influence on the redness of the roots, than the yellowness. This is in accordance with Zude *et al.* (2007) report, who found very weak relationship between carotenoids in carrot roots and the CIE colour parameters of the roots. They found that a* was the strongest correlated with carotenoids

Tab. 7. Colour parameter a* of carrot juice, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	22.6	24.6	22.3	24.3	21.8	24.9 ^a
60 + 0	21.8	22.3	19.7	22.7	22.4	26.3 ^b
30 + 30	22.8	22.7	22.5	22.5	22.7	26.1 ^b
0 + 60	23.3	22.4	23.7	22.6	23.8	24.4 ^a
0	24.7	21.9	22.0	21.0	22.1	24.9 ^a
Means for cultivars	23.1 ^b	22.8 ^{ab}	22.0 ^a	22.6 ^{ab}	22.6 ^{ab}	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

Tab. 8. Colour parameter b* of carrot juice, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	28.3	31.6	31.1	30.0	28.3	30.3 ^a
60 + 0	27.7	29.4	28.0	28.4	29.0	31.5 ^a
30 + 30	28.8	29.6	30.7	27.5	28.8	31.5 ^a
0 + 60	28.6	30.5	30.4	27.8	29.9	30.5 ^a
0	30.4	29.8	30.4	27.1	28.7	30.0 ^a
Means for cultivars	28.7 ^a	30.2 ^b	30.1 ^b	28.1 ^a	28.9 ^a	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

Tab. 9. Dry matter of carrot roots, in relation to N fertilization and cultivars

N dose (kg/ha)	'Karotan'	'Trafford'	'Krakow'	'Komarno'	'HY 7842'	Means for N dose
120 + 0	12.75	14.25	10.25	10.70	11.15	11.82 ^b
60 + 0	11.40	12.25	10.60	9.60	11.00	10.97 ^a
30 + 30	11.80	12.60	10.55	10.55	8.90	10.88 ^a
0 + 60	13.45	14.10	12.05	9.30	10.75	11.93 ^b
0	13.20	12.45	14.00	9.65	11.20	12.10 ^b
Means for cultivars	12.52 ^c	13.13 ^c	11.49 ^b	9.96 ^a	10.60 ^a	

Note: means for factors which do not differ according to Tukey's HSD test at P = 0.05 are marked with the same letters

Also Skrede *et al.* (1997) reported that carrot roots rich in carotenoids showed a more reddish colour. In our study the relationship between the a*

Tab. 10. Correlation coefficients r between carotenoid compounds content in carrot roots and colour parameters of roots, juice and dry matter

Parameter	Total carotenoids	β -carotene
L* - roots	-0.09	-0.08
a* - roots	0.50**	0.49**
b* - roots	-0.15	-0.14
L* - juice	-0.32*	-0.21
a* - juice	0.42**	0.41**
b* - juice	0.02	0.03
Dry matter	-0.43**	-0.42**

Note: ** means significant correlation at P=0.05, * means significant correlation at P=0.10

parameter of the roots and the carotenoids content was a little stronger than between the a* parameter of the juice and the carotenoids content.

Conclusions

Total carotenoids and β -carotene accumulation in carrot roots was related to carrot genotypes. The cultivar of the highest carotenoids content was the experimental cultivar 'HY 7842'.

Carotenoids accumulation in the carrot roots was insignificantly affected by nitrogen fertilization at the rates applied in the study.

The colour parameter a* for carrot roots and juice was moderately strongly correlated with the content of carotenoid in the roots. However, the L*, a* and b* parameters of the roots and juice were insignificantly correlated with nitrogen fertilization rates.

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