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Carotenoids of a New Russian Carrot Cultivar F1 "Rubinovaya"

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***Abstract*—New Russian F1 "Rubinovaya" cultivar of Daucus carota was found to be pink-colored due to biosynthesis of lycopene and β-carotene in relatively high overall level (up to 0.200 mg per 1 g of fresh weight, FW). The typical carotenoid composition of carrot roots with pink color were determined by proposed in the paper reversed-phase HPLC method. These are all-trans-lycopene (28 - 47 %); mixture of cis-lycopene isomers (7 – 10 %), all-trans-β-carotene (30 - 55%) and mixture of cis-β-carotene isomers (7 – 10 %). From chromatographic point of view carotenoids are retained in the proposed conditions by distribution mechanism due to a long chain of conjugated C=C-bonds makes this part of carotenoids planar with ability penetrate space between grafted C18 chains.**

Keywords—Daucus carota, R1 "Rubinovaya" cultivar, lycopene, β-carotene, RP HPLC, distribution mechanism of retention formatting, style, styling, insert

I. INTRODUCTION

In addition to the usual varieties of orange carrots, which accumulate β- and α-carotene, as important compounds with provitamin A activity, in recent years, more attention is paid to varieties with the accumulation of other carotenoids and even with anthocyanins [1]. These substances include lutein that is necessary for prevention of age-related macular degeneration, AMD [2], and lycopene as one of the most potential antioxidant [3] of carotenoid nature. The corresponding carrot varieties differ from the usual varieties by the color, appearing as yellow and red correspondingly.

Asia Minor and the inner Asiatic regions were the origin centers of cultivated carrots [4]. In addition, regions including Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan were the basic centers of cultivated carrots in Asia. The roots of the plant were purple and yellow colored [5]. These carrots spread east and west from this center to the Middle East, North Africa, Europe, and China in the XVth century. Until the development of orange carrots in The Netherlands (18th century) yellow-colored carrots were cultivated in northern Europe. Later purple and yellow carrots remain ordinary only in some areas of Turkey, India, and China while red carrots in Japan [1].

The high biological activity of lycopene [3] – the most advertised carotenoid in recent years has led to a great interest in plant sources of this substance. To date, the most important sources of lycopene of the Belgorod region traditional plants are tomatoes and watermelons (both with red colored flesh). The other sources are not so well known and popular, including some species of rose hips, *Momordica charantia*, gumi-fruit (*Elaeagnus multiflorum*), alpine currant (*Ribes alpinum*), etc.

Meanwhile carrots that synthesize lycopene remain almost unknown in Russia. Until recently seeds of the only unique carrot variety "Atomic Red" were hardly available due to non-professional gardeners. Only this year new hybrid carrots F1 "Rubinovaya" with an unusual pink color appeared on the market.

The determination of carotenoid composition of this hybrid was the task of a current investigation.

II. EXPERIMENTAL

Sample preparation

Carrots were grown by direct seed sowing in the late April of 2019 and harvested in late September in three different parts of Belgorod. After harvesting, the roots were washed with water, and after water excess were stored in refrigerator covered with paper.

Extract No. 1 preparation. Carrots were cut into pieces and homogenized in porcelain mortar, frozen and lyophilized. Carotenoids from the sample of the resulting plant material were extracted with some portions of acetone in porcelain mortar under a solvent when rubbed with a pestle. Extractions was proceeded until receiving colorless extract. Extract portions were combined in a volumetric flask, the volume was made up by acetone for spectrophotometric analysis.

Extract No. 2 preparation. For HPLC analysis extract No. 1 was transferred into the separating funnel. After addition of *n*-hexane and saturated aqueous solution of NaCl

the carotenoids were transferred into *n*-hexane. The *n*-hexane was withdrawn on a vacuum rotary evaporator, the residue was dissolved in a mobile phase and filtered through syringe filter CHROMAFIL®Xtra (0.45 μm).

Spectrophotometric analysis

Extract No. 1 was proper diluted with acetone to reach optical density in the region 0.1 – 1.1. The optical density of the solutions was determined in the quartz cuvettes at 472 nm in the case of electronic absorption spectra type I and at 450 nm in the case of electronic absorption spectra type II, Fig.1.

The calculations of overall carotenoids content (as mg per 1 g of FW) were performed by equation 1:

$$\propto = \frac{A \cdot V_{E1} \cdot D}{E_{1\ cm}^{1\ \%} \cdot l \cdot m} \cdot 1000, \text{(mg per 1 g)} \qquad (1)$$

where A – optical density; V_{E1} – volume of extract No. 1, l; D – degree of dilution;; $E_{1\ cm}^{1\ \%}$ – extinction coefficient for analytical wave length (3466 for 472 nm for β-carotene [6]); l – cuvette pass length, cm; m – mass of the carrot sample, g.

Reversed-phase HPLC

An Agilent Infinity 1260 liquid chromatograph, equipped with a diode array detector, was used for the analysis. The data were collected by Agilent ChemStation.

Sample (10 ml) was injected onto 4.6×100 mm Kromasil 100-5C18 (the main column); 4.6×100 mm Kromasil 100-5C8 and 4.6×100 mm Kromasil 100-5C4 columns at column thermostate temperature 30°C.

Mobile phase contained mixture of acetone (Fisher chemicals) and acetonitrile (LiChrosolv®, gradient grade for liquid chromatography); flow rate: 0.4 ml/min. Chromatograms were recorded at 472 nm.

Chromatograms and electronic absorption spectra of the carotenoids were exported in MSExcel for further processing and figures drawing.

For correct determination of fraction of carotenoids with different electronic absorption coefficients and different extinction coefficients, the commonly used equation 2 is not applicable.

$$(A) = \frac{S(A)}{\sum_i S(i)} \cdot 100, \% \qquad (2)$$

Corrected peak normalization procedure

For correct determination of fraction of carotenoids with different electronic absorption coefficients and different extinction coefficients, the commonly used equation 2 is not applicable.

$$\alpha(A) = \frac{S(A)}{\sum_i S(i)} \cdot 100, \% \qquad (2)$$

where $\alpha(i)$ is a %-fraction of compound A in a mixture, S(i) and $\sum_i S(i)$ are peak area of sample i and the total sum of all compounds areas on the chromatogram. Instead of the equation 2 we propose equation 3 with correction factors, $k(i)$.

$$\alpha(A) = \frac{k(A) \cdot S(A)}{\sum_i k(i) \cdot S(i)} \cdot 100, \% \qquad (3)$$

Correction factors may be calculated by the following approach. The chromatogram must be registered at a specific for one compound (of the mixture) wavelength. Thus, a correction coefficient for this substance (reference) may be taken as 1. Then the ratio of extinction coefficients (ε) at two wavelengths (at λ_{max} and at λ of chromatogram registration) for compound A must be multiplied by the ratios of extinction coefficients ($E_{1\ cm}^{1\ \%}$) of reference compound (ref.) and compound A, equation 4.

$$k(A) = \frac{\varepsilon(A, \lambda_{max}(A))}{\varepsilon(A, \lambda_{reg}(A))} \cdot \frac{E_{1cm}^{1\%}(\lambda_{max}(ref.))}{E_{1cm}^{1\%}(\lambda_{max}(A))} \qquad (4)$$

III. RESULTS AND DISCUSSION

Choice of reversed-phase stationary phase

In the case of "monomeric" alkyl grafted phases the choice of stationary phase type depends upon the retention mechanism of the solutes to be separated. By a method described elsewhere [7] it is necessary for a given mobile phase to determine the change of solute retention when C18-phase is substituted by C8-phase and then replaced by C4-phase. If the retention markedly drops it may be concluded that distribution mechanism is responsible for solutes retention. Indeed, it was the case of carotenoids that were late identified as β-carotene and lycopene separation, Fig.2.

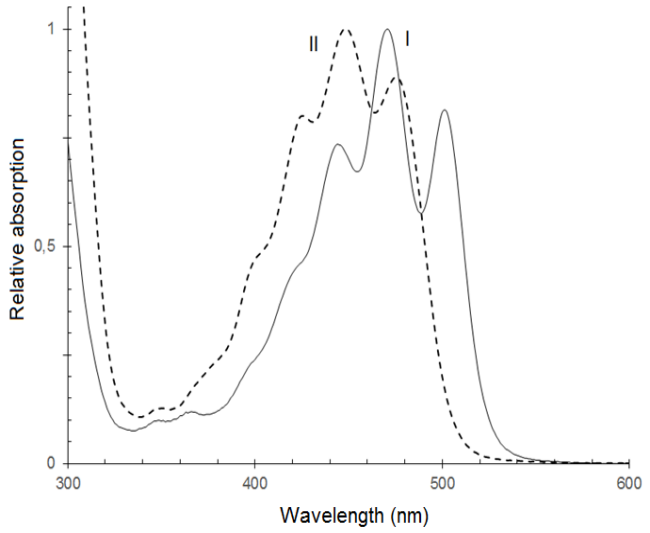

Fig.1. Two types of electronic absorption spectra of F1 "Rubinovaya" carrot cultivar acetone extracts: I – of carrots with pink color and II – of that with ordinary orange coloration

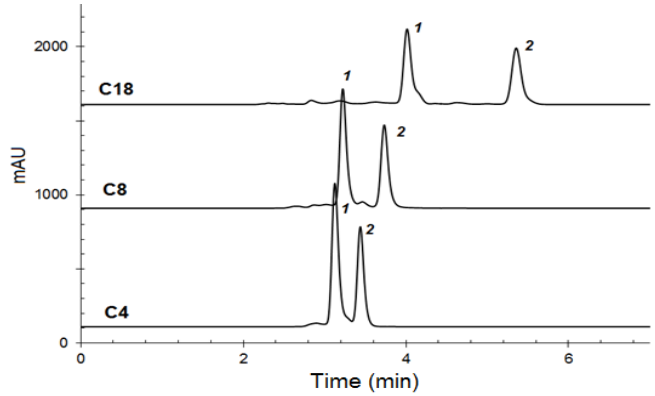

Fig.2. Retentions of lycopene (1) and β-carotene (2) upon three different stationary phases (C18, C8 and C4). Mobile phase: acetone 70 vol. % and acetonitrile 30 vol. %. Other conditions see Experimental part.

Thus for separation of β-carotene and lycopene C18-phase is preferable because of solubility properties of the carotenoids demanding high concentration of acetone to be present in a mobile phase.

Choice of mobile phase composition

The concentration of acetone in a mobile phase of a mixture with acetonitrile may be chosen to permit separation of the main carotenoids of the samples under investigation at a reasonable time of compound elution. For the three concentrations of acetonitrile in mobile phases results of orange carrot carotenoid separation are presented in the Fig.3.

Thus for separation of β-carotene and lycopene C18-phase is preferable because of solubility properties of the carotenoids demanding high concentration of acetone to be present in a mobile phase.

An increase of acetonitrile content to 50 vol. % permits separation of all-trans α-carotene, all-trans β-carotene and sum of its cis-isomers, that are commonly found in carotenoids mixtures of orange-colored carrot. The further increase of acetonitrile concentration to 60% is senseless because of increase of retention of all compounds without improvement of solutes separation. Thus, a concentration of 50 vol. % of acetonitrile and 50 vol. % of acetone was the mobile phase composition utilized for the further chromatographic investigations.

Results of spectrophotometric carotenoid determination

Electronic absorption spectra of carrot acetone extract indicated that for some cases β-carotene remained the main carotenoid. Thus, hybridization was not always perfect and some orange-colored carrots still may be harvested. However, pink colored carrots were found in the most cases and even electronic absorption spectra of the carotenoid extracts revealed lycopene biosynthesis for F1 “Rubinovaya” carrot cultivar. Overall content of carotenoids calculated as prevalent carotenoid equivalent is presented in Table I; it is in agreement with literature data for the carrots [8].

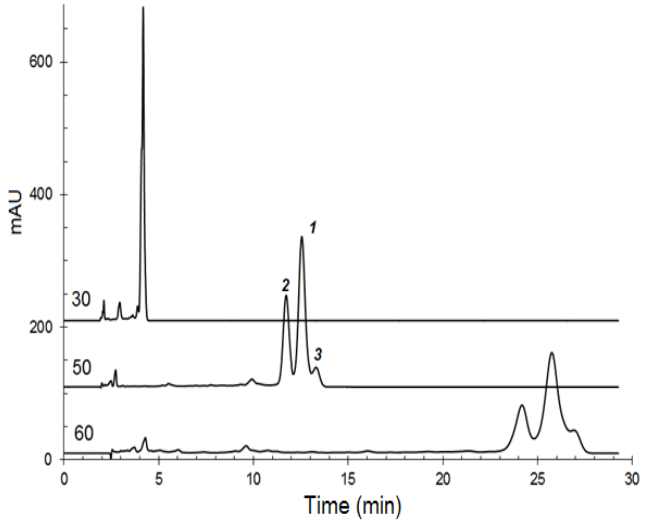

Fig.3. Separation of orange carrot carotenoids at three compositions of a mobile phase. Acetonitrile vol. fractions (%) is pointed over the chromatograms. Substances: 1 – all *trans* β-carotene; 2 – all-*trans* α-carotene; 3 – mixture of *cis*-isomers of β-carotene.

TABLE I. CAROTENOID COMPOSITION OF F1 “RUBINOVAYA” CARROT SAMPLE ROOTS

№	Total carotenoid content	Mole fractions of carotenoids, %, ± 0.3 %						
		lutein	*lycopene*		*α-carotene*	*β-carotene*		*Other carotenoids*
			trans	*cis*		*trans*	*cis*	
1	0.106*	2.5	0	0	32.7	54.1	7.1	3.6
2	0.188**	0.9	28.8	7.1	0	53.4	3.7	7.0
3	0.121**	5.4	47.1	7.1	0	29.6	3.3	7.6
4	0.152**	1.2	41.7	9.9	0	38.0	4.3	6.0
5	0.205**	0.8	35.9	9.6	0	43.9	5.7	5.0

* - expressed as β-carotene equivalent; ** - expressed as lycopene equivalent, mg per 1 g of FW.

RP HPLC of carotenoids

Chromatograms of acetone extracts of 5 samples of carrot F1 ”Rubinovaya” are presented in the Fig.4.

Sample No.1 has a carotenoid composition of an ordinary orange colored carrot [9], including all-*trans* β-carotene and sum of its *cis*-isomers and α-carotene. These compounds are easily identified by electronic spectra, Fig.5, when compared to literature data [10]. β-carotene has a maximum of absorption at 450 nm. The band is shifted hypsochromically by 4-5 nm with appearance of characteristic *cis*-band at 320 – 360 nm. Band for α-carotene is also hypsochromically shifted due to exclusion of the ring double bond from conjugation with C=C-bonds of central part of carotenoids, Fig.5.

Meanwhile the other four carrot samples were unique due to distinct biosynthesis of lycopene, though the content of β-carotene is still valuable but α-carotene has disappeared at all, Fig.4. All-*trans* lycopene and it *cis*-isomers are readily distinguished by electronic spectra presented in the

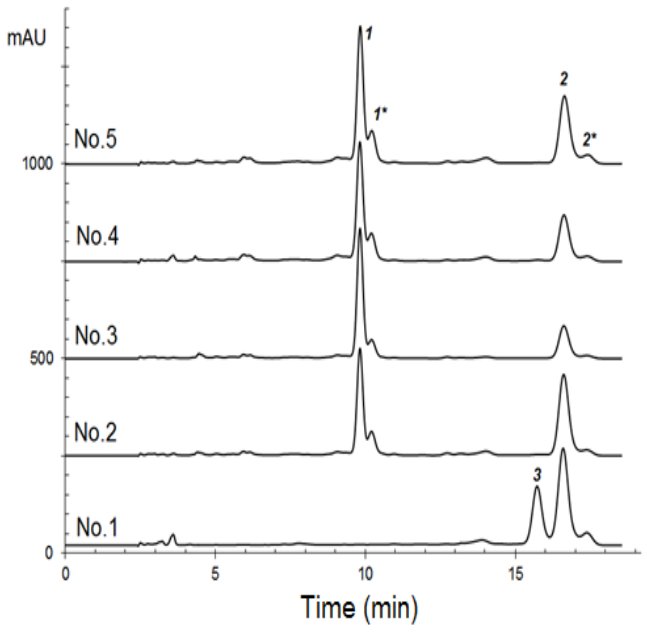

Fig.4. Chromatograms of 5 samples of carrot cultivar F1 “Rubinovaya” acetone extracts. Carotenoids: 1 – all-*trans* lycopene; 1* – sum of *cis* isomers of lycopene; 2 – all-trans β-carotene; 2* – sum of *cis* isomers of β-carotene; 3 – α-carotene.

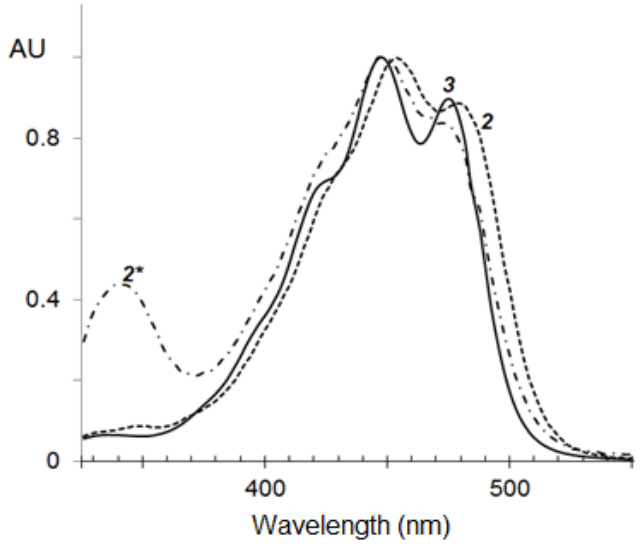

Fig.5. Electronic absorption spectra of sample No.1 (Fig.4) carotenoids. Number of bands correspond to peak numbering in the Fig.4

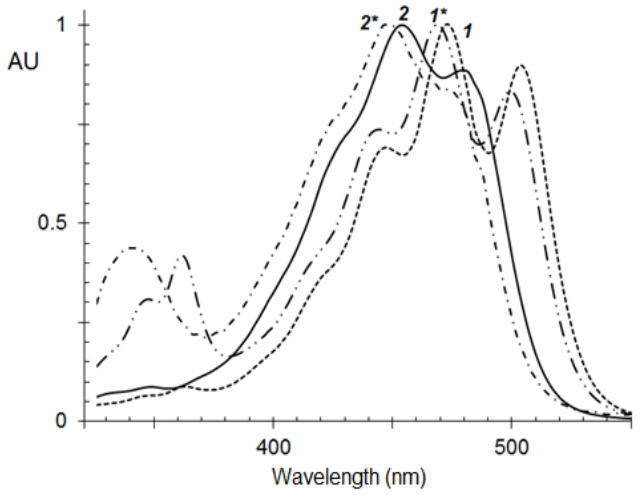

Fig.6. Electronic absorption spectra of carotenoid types in extracts of carrot samples No.2 – No.5 in the Fig.4. Number of bands correspond to peak numbering in the Fig.4.

Indeed electronic absorption spectra of all *trans*-lycopene (band 1) has the biggest wavelength value of λ_{max} due to a perfect conjugation of C=C-bonds. The band moves hypsochromically for mixture of *cis*-lycopenes (band 1*). The positions of β-carotene isomers' λ_{max} were discussed above.

Some remarks on carrot roots colorant composition

Thus, new F1 cultivar "Rubinivaya" is a first investigated Russian cultivar of *Daucus carota*, with moderately expressed biosynthesis of lycopene with still high content of β-carotene. This cultivar combines high content of the most powerful lipid soluble antioxidant with another valuable carotenoid – β-carotene with provitamin A function. Moreover, uncommon pink (not red) the color of the roots was the consequence of one more colorant – but not of carotenoid origin. Due to acidified (0.1 M HCl) water extraction with subsequent extract purification and RP HPLC analysis some small quantities of anthocyanins were found with qualitative composition closed to those published in literature [11].

The overall carotenoid accumulation calculated as β-carotene (sample No.1) equivalent of (for samples No. 2 - 5) as lycopene content was relatively high in comparison with published in literature data.

IV. CONCLUSIONS

Rose-colored F1 "Rubinovaya" *Dacus carota* cultivar is the first investigated Russian cultivar with expression of lycopene biosynthesis together with β-carotene. The high overall carotenoid accumulation makes this cultivar very promising for direct utilization as a food component, as well as a source for lycopene-colorants preparation.

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