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INFLUENCE OF RELATED COMPOUNDS ROOT RED CARROT ON SOLUBLE CAROTENOIDS

The thermotreatment of the domestic carotenoid-containing plant materials of the sowing carrot roots *Daucus sativus* Roehl allowed to get the concentrate of hydrophilized carotenoid compounds used in the food industry as a colorant. The effect of the attendant compounds on the hydrophobic-hydrophilic properties of carotenoids was studied on the model system of the main components of the sowing carrot roots using spectrophotometry and HPLC.

Introduction. Natural and synthetic food dyes are used for foodstuffs painting in some sectors of the food industry [1].

The use of natural dyes produces allows to obtain not only painted foods, but also enriched with biologically active compounds. It happens because many natural pigments exhibit some properties of vitamins, antioxidants, radiation protectors [2].

β -carotene (provitamin A) and certain of its derivatives such as xanthophylls at different oxidation play an important role among the natural pigments. Concentrates of carotenoids extracts, derived from natural vegetable raw materials are currently used as natural dyes. In Russia and other countries with temperate latitudes climate the major natural carotenoid pigment is β -carotene (Fig. 1). Pigment sources are pumpkin, carrots, spinach, tomatoes, red peppers, plums, gooseberries, blueberries, black currants.

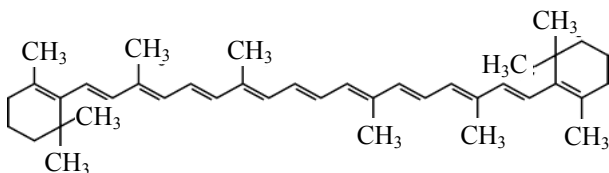


Fig. 1. Structural formula of β -carotene

Carotene molecule chemical structure is an unsaturated hydrocarbon polyene with general formula of $C_{40}H_{56}$, exhibiting hydrophobic characteristics, so these pigments are extracted by non-polar extractants only from plant raw material. Extracts concentrates may be used for painting the fat-containing foods.

Highly oxidized carotenoids with carboxyl groups and carbohydrate molecules in their composition are contained in the plant material in tropical countries due to thermal-oxidative processes. For example, the main pigment of annatto extract, seed of Orellana tree *Bixa orellana* L., is carotenoid bixin $C_{25}H_{30}O_4$, and an extract pigment of saffron plant *Crocus sativatus* L. is krotsetin $C_{20}H_{24}O_4$ with two molecules of a disaccharide in the form of crocin (diester gentsibiozy) [3]. Hydrophilic carotenoids are readily soluble in alcohol and water and are used for water-based food coloring.

Main part. Our research has focused on the search for import-substituting water-soluble carote-

noid pigments, as well as the development of methods for β -carotene hydrophilizing, which is the main pigment of such widespread raw material in Russia and Belarus as carrot-roots *Daucus sativus* Roehl [4, 5].

Clean raw material was crushed and dried at room temperature under diffuse illumination throughout the day and night, and then extraction was performed.

The study of the efficiency of the extraction separation of carotinoids from carrot roots showed a significant role of the organic solvent as well as the influence of the related compounds presented in raw materials.

Researches analyzing the extraction of natural carotinoids of carrot-root showed that the extraction of the pigments by aprotic low-polar solvents such as ethyl acetate or carbon tetrachloride takes place without extraction of raw materials of related compounds. The final extract had a red-orange color with a characteristic electronic absorption spectrum for β -carotene. Absorption peaks were observed at 428, 450 and 474 nm.

A clear, yellow extract was derived in the process of the extraction of the pigments of just reduced carrot-roots by aqueous ethanol solution with a volume fraction of 96% of ethanol. Absorption maxima in the zone of 360–500 nm were absent. The following dilution of the extract with 96% aqueous ethanol solution led to the clouding of the solution. Apparently, the extracting of hydrophobic β -carotene with 96% aqueous ethanol solution happened in the complex (associate) with hydrophilic hydroxyl molecules of the components of carrot. Absence of an absorption maximum in the electronic spectrum of β -carotene shows the difficulty of forming of photo-excited polyene structure due to participation in the complexation of electron π -bonds. Dilution of the extract with 96% aqueous ethanol solution destroys the carotene hydrophilic complex to precipitate the free molecules of hydrophobic carotene.

In order to study the impact of major chemical (related) compounds of carrot roots (*Daucus sativus* Roehl) the model mixture of carrot major components in the following proportions: β -carotene – 0.01 g; dextrose – 2.5 g; sucrose – 3.5 g; pectin –

0.6 g; malic acid – 0.2 g; aspartic acid – 0.14 g; glutamic acid – 0.24 g was prepared [5].

The spectral methods are used for quantitative analysis of β -carotene. Ethylene system of conjugated double bonds is a carotenoid chromophore group, which determines the color of carotenoids [6]. Spectrophotometric analysis of aqueous-ethanol extracts of carotenoid pigments from the obtained mixture with different volume ethanol content (96% and 48%) showed that β -carotene (determined by absorbance at $\lambda = 440$ nm) and related compounds (determined by absorbance at $\lambda = 364$ and 400 nm) are greater than the extract obtained by using 96% aqueous ethanol solution as compared to 48% ethyl solution, other things being equal (Fig. 2). For example, for the extraction of 96% aqueous ethanol solution the following ratio were used: $A_{440} / A_{400} = 0.58 / 0.95 = 0.61$, $A_{440} / A_{364} = 0.58 / 2.0 = 0.29$, and for 48% aqueous ethanol solution $A_{440} / A_{400} = 0.16 / 0.31 = 0.52$, $A_{440} / A_{364} = 0.16 / 0.95 = 0.17$. It is explained by lower polarity and higher extraction capacity of β -carotene associates with hydrophilic components of carrot model mixture.

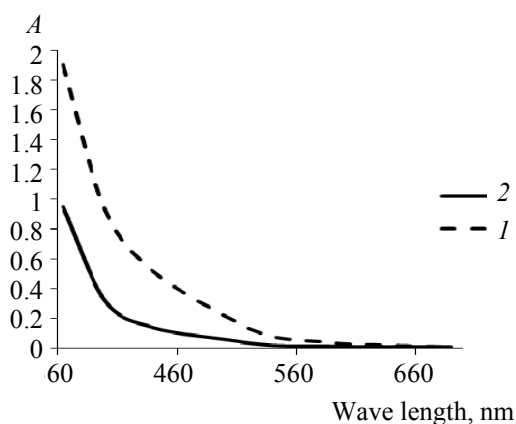


Fig. 2. Range of water and ethanol extracts with different ethanol fraction of the model mixture of components of carrot roots:
1 – volume fraction of ethanol is 96%;
2 – volume fraction of ethanol is 48%

It is interesting to note that the content of β -carotene in a water-saturated ethanol solution of ethanol with a 96% volume fraction is less to be compared with the content of β -carotene in carrot model mixture components. It confirms the influence of polar related substances contained in carrot roots on solubility of carotenoids.

In the process of addition of β -carotene (β -precipitate having carotene) pectin solution to 96% saturated aqueous ethanol solution (in a ratio of 1 volume of solution to 1 volume of carotene pectin solution) the β -carotene content in the resulting mixture increases. For example, carotene content has increased by 1.36 times when added a saturated alcoholic solution of pectin with 70% degree

of esterification to 96% β -carotene ethanol solution saturated (with β -carotene precipitate).

After the gradual heat treatment of model mixture basic components of carrot root in the air at $t = 40^\circ\text{C}$ ($\tau_1 = 2$ hours), $t = 60^\circ\text{C}$ ($\tau_2 = 2$ hours), $t = 80^\circ\text{C}$ ($\tau_2 = 2$ hours) (Fig. 3) the carotenoids content in the extract of 96% aqueous ethanol solution was not substantially changed, and the carotenoid content in the composition of the ethanol extract with a volume fraction of 48% ethanol was increased on an average of 1.15 to 1.35 times ($A_{440} / A_{400} = 0.15 / 0.25 = 0.60$; $A_{440} / A_{364} = 0.15 / 0.66 = 0.23$ for the 48% ethanol extract of the model of carrots after heat treatment; $A_{440} / A_{364} = 0.16 / 0.95 = 0.17$ ethanol extract from the model of carrots without heat treatment).

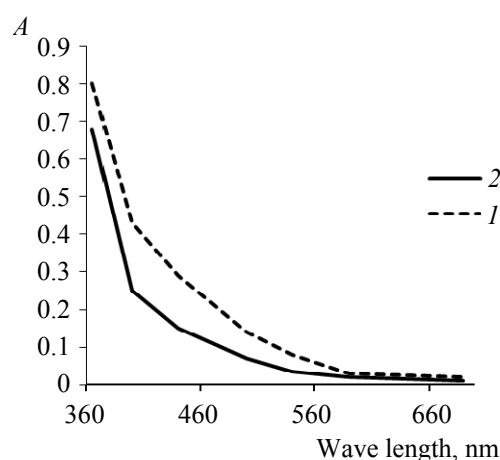


Fig. 3. Range of water and ethanol extracts with different ethanol fraction of the model mixture of components of carrot roots after gradual heat treatment:
1 – volume fraction of ethanol is 96%;
2 – volume fraction of ethanol is 48%

Gradual heat treatment provides a dye mixture with lower energy costs in the conditions that enhance the safety of β -carotene in the raw materials and minimize the formation of secondary pigment-melanoidins [4, 7].

The observed effect is not obviously associated with increasing concentration of more oxidized forms of carotenoids, but also a higher concentration of carotenoids associates with the heat-treated pectin.

Investigation of the spectral characteristics of water-ethanol extracts having a 96% of ethanol content of the pectin before and after the heat treatment showed an increase of concentration of the compounds of low molecular weight pectin.

Chromatographic studies using HPLC confirmed spectrophotometric data related to the carotenoid content increase in an aqueous ethanol solution in the presence of pectin. Moreover, the heat treatment with β -carotene pectin contributed to increasing the carotenoid content of aqueous ethanol solutions (Fig. 4).

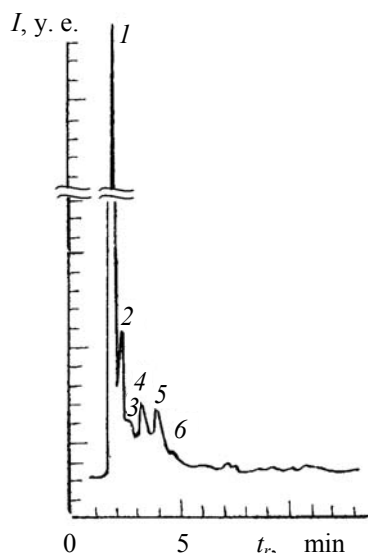


Fig. 4. HPLC carotenoid extract chromatogram (96%) of the heat treated carrot roots:
 1 – carotene, ($t_r = 2.0$ min, content – 74.8 wt d. %);
 2–6 – carotenols (t_r 2.5, 3.0, 3.7, 4.2, 4.6 min respectively, content – 12.0, 4.4, 5.4, 2.0, 1.4 wt d. % respectively)

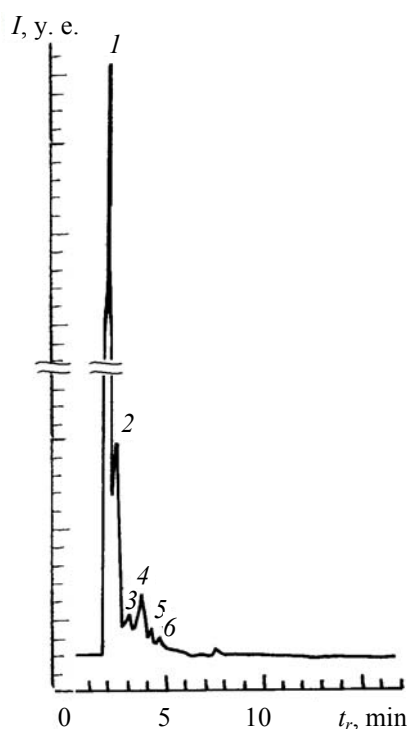


Fig. 5. HPLC carotenoid extract chromatogram (96%) of the model mixture:
 1 – carotene, ($t_r = 2.0$ min, content – 58.7 wt d. %);
 2–6 – carotenols (t_r 2.3, 2.7, 3.3, 4.0, 4.7 min respectively, content – 6.8, 7.1, 10.3, 11.1, 6.0 wt d. % respectively)

Chromatographic parameters: stationary phase – Separon SGX; mobile phase – heptane: isopropanol in a volume ratio of 99.9 : 0.1; eluent flow rate of $0.5 \text{ cm}^3 / \text{min}$; detection at $\lambda = 436 \text{ nm}$.

Chromatographic analysis confirms that with the presence of hydrocarbons as well as oxygen containing carotenoids (carotenols) the process of pigments extracting by aqueous ethanol solution (96%) from plant raw material results in the formation of an extract, containing as the hydrophobic carotenoids $\text{C}_{40}\text{H}_{56}$ as more hydrophilic fitoksanantins $\text{C}_{40}\text{H}_{56}\text{O}$, $\text{C}_{40}\text{H}_{56}\text{O}_2$, $\text{C}_{40}\text{H}_{56}\text{O}_3$. It is confirmed by chromatographic analysis (Fig. 5).

Conclusion. Thus, our studies suggest the increasing of the hydrophilic properties of natural carotenoid pigments under the reaction of thermal oxidation of biological systems. It happens because of the complex formation of pigments consisting of 75–60 wt d. % of the hydrophobic β -carotene and the 25–40 wt d. % of xanthophylls such as $\text{C}_{40}\text{H}_{56}\text{O}$ (lutein and zeaxanthin), due to the appearance the hydroxyl groups of the polar compounds in the structure of these molecules. This process is associated with a possible pectin- β -carotene-fitoksanantins formation.

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