Investigation of the potential of industrial carrot processing waste for the release of bioactive substances

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Abstract. Bioflavonoids have anti-inflammatory, antitumor, neuroprotective, antidiabetic, antimicrobial, antithrombogenic activity. A large amount of these valuable bioactive substances gets wasted after processing of fruits and vegetables, carrots in particular. The article studies the possibility of using carrots pulp of the Losinoostrovskaya, Nantes (Nantskaya) and Queen of Autumn (Koroleva Oseni) varieties as a source of flavonoids and carotenoids. It was determined that the highest content of the target substances is extracted using ethanol with a concentration of 96%. We carried out the pectrophotometric evaluation of the composition and quantitative determination of flavonoids and carotenoids in pulp. It was found that carotenoids were the predominant component of extracts in the studied pulp samples. Their content was 1.8-1.9 times higher than flavonoids. One of the directions in the development of methods for increasing the efficiency of isolation of bioactive compounds can be methods for increasing the yield of bound phenolic compounds with high biological activity.

1. Introduction
Currently, special attention is paid to the development and application of raw materials with functional properties aimed at enriching the composition of the finished product with micro and macro elements, essential amino acids, dietary fiber and antioxidants [1, 2]. The main task of the latter is the inactivation of free radicals in the human body [3, 4].

The main antioxidants in plant materials are bioflavonoids, as well as carotenoids. In recent decades, these substances have also been identified to have anti-inflammatory, antitumor, neuroprotective, antidiabetic, antimicrobial, antithrombogenic activity of flavonoids, their effectiveness in various cardiovascular, pulmonary and other diseases. This is especially attractive due to the high availability and relative cheapness of food flavonoids. Over time, all noted types of pharmacological activity were confirmed in vitro using cell cultures and a variety of model systems [5, 6, 7].

In addition to the positive effect exerted by flavonoids on physiological processes and human health, they can be used in the food industry to extend shelf life and replace synthetic antioxidants used for this purpose [8]. In this connection, they are actively searching for their sources and methods of isolation [9, 10].

The priority direction in the development of the food industry is the non-waste production and use of components of the raw material base with high technological properties and low cost, allowing to
expand the assortment, increase the nutritional value of the products, reduce the cost of manufacturing finished products and reduce the amount of technological waste.

Production waste remaining after processing fruits and vegetables, which can serve as sources of bioactive substances, are individual copies of substandard raw materials and pulp. In large quantities, waste is generated in the production of fruit drinks, juices, marshmallows, etc. (tab. 1). As can be seen from the table, one of the largest amounts of waste is formed upon receipt of carrot juice.

Table 1. The proportion of waste in various processes

| Technological process               | The amount of waste, % |
|------------------------------------|------------------------|
| Getting Apple Juice                | Up to 40               |
| Getting Apple sauce                | 12                     |
| Getting apple jam (with peel)      | 24                     |
| Getting apple jam (without peel)   | 30                     |
| Getting carrot juice               | 35                     |
| Peeling onions                     | 17                     |
| Getting Pickled Horseradish        | up to 45               |
| Pumpkin peeling                    | 30                     |
| Pumpkin                            | 19                     |
| Getting natural canned food        | 10                     |
| Getting Tomato Sauces              | 12.5                   |
| Getting Tomato Juice               | 35                     |
| Getting pastilles (from sea buckthorn) | 20                  |

An analysis of previous studies showed that carrots growing in different regions contain different amounts of flavonoids and carotenoids with high antioxidant activity [11, 12], but there is no similar information regarding carrots grown in the Russian Federation in the Kemerovo region.

The aim of the study was to assess the possibility of using carrot pulp as a source of bioactive substances.

2. The object of the study
We used carrots varieties "Losinoostrovskaya", "Nantes" (Nantskaya) and "Queen of Autumn" (Koroleva Oseni), zoned in the Siberian Federal District, harvest year 2019, harvested in the Kemerovo region. The pulp used in research is waste, which was received from the enterprise after the production of carrot juice.

3. Materials and methods
Pulp, as an object of study, was obtained from an industrial enterprise and was used without additional processing. Until the time of the study, it was stored for no more than 1 hour.

The moisture and volatiles content was determined by the thermogravimetric method at a temperature of 105 °C for 2 hours [13].

The preparation of solutions of the extractives of the samples was carried out as follows: about 2.0 g (accurately weighed) of the crushed raw material was placed in a flat-bottomed flask with a capacity of 100.0 ml and 50.0 ml of solvent and weighed with an accuracy of ±0.01 g. The flask with the contents was brought to a boil in a water bath under reflux for 2 hours, periodically shaking. We cooled the obtained extract, and adjusted the mass with the solvent to the original. The resulting extract was filtered through an ashless filter (solution “A”). An aliquot of solution “A” 5.0 ml was placed in a volumetric flask with a capacity of 50.0 ml and the volume of the solution was adjusted to the mark with the used solvent (solution “B”). The following solvents were used: water, ethyl alcohol solution (40%), ethyl alcohol solution (70%), ethyl alcohol (96%).
Quantitative determination of flavonoids was carried out according to the following procedure [14, 15]: We used an extract based on a solvent of ethyl alcohol (96%) was used for determination. 1.0 ml of solution "B" was placed in two tubes. We first added 0.3 ml of a 5% solution of sodium nitrite to the first tube, after 5 minutes, we added 0.3 ml of a 10% solution of aluminum chloride in ethyl alcohol. Finally, after 6 minutes, we added 2 ml of 1M sodium hydroxide and 2.4 ml of distilled water. Only 5 ml of distilled water was added to the second tube. After 20 minutes, the optical density of the solution was measured on an SF-2000 spectrophotometer at a wavelength of 510 nm in cuvettes with a layer thickness of 10 mm.

As a comparison solution, we chose a solution from a second tube. The standard curve was built on the basis of solutions of catechin hydrate of various concentrations. The total flavonoid content was expressed in milligrams of catechin equivalent per 100 grams of dry weight of the sample.

The content of carotenoids was determined by the following method: For determination, we used an extract based on a solvent of ethyl alcohol (96%). The optical density of the extract (solution “B”) was measured at a wavelength of 450 nm in a cuvette with a layer thickness of 10 mm, using ethyl alcohol 96% as a comparison solution.

The content of carotenoids in the sample, in terms of β-carotene, in milligrams per 100 grams of dry weight of the sample is calculated by the formula:

\[ X = \frac{D \times 50 \times 5 \times 100 \times 100}{2500 \times 5 \times m \times (100 - W)} \]

where D is the optical density of the test solution at a wavelength of 450 nm;

m is the mass of the sample, in grams;

W is the loss in mass upon drying, %;

2500 – specific absorption rate of β-carotene.

Extracts were prepared in a single repetition. The content of carotenoids and flavonoids was measured in triplicate. The results were processed using Microsoft Office 2007 software. The average values and standard deviation of the results are presented in the article.

4. Discussion of the results
As you know, carrots contain a significant amount of both non-polar carotenes (α- and β-carotene), and polar xanthophylls (lutein). In addition to carotenoids, flavonoids are a valuable component of carrots, and 30-90% alcohol is usually used to extract them. For a preliminary assessment of the composition of extractable bioactive compounds from carrot pulp of the Losinoostrovskaya variety, extraction was performed with various solvents. The obtained extracts were determined UV spectra, which are presented in Figure 1.

An analysis of the data presented shows that there are spectra characteristic of α-, β-carotene and lutein with pronounced absorption maxima at 450 and 480 nm. Another maximum at 270-280 nm is also characteristic of carotenoids and various bioflavonoids. It can be seen that the maximum amount of target substances is extracted using ethanol 96%. It can be noted that in the spectra of pulp extracts in ethanol 70% and 96% there are no pronounced maxima in the region of 320-360 nm. These absorption bands can produce bioflavonoids, such as luteolin, quercetin, rutin, which are found in carrots [12].

The share of moisture and volatile substances of the pulp of carrots of the varieties "Losinoostrovskaya", "Nantes" (Nantskaya) and "Queen of the Autumn" (Koroleva oseni) amounted to 84.3 ± 0.3%, 83.1 ± 0.4% and 83.5 ± 0.4%, respectively.

For the quantitative determination of carotenoids and bioflavonoids in pulp of carrots of various varieties, extraction was performed with 96% ethanol. The determination was carried out by photocolorimetric methods according to the above methods. The results of the determination are presented in table 2.
Figure 1. UV spectrum of carrot pulp extracts obtained by different solvents: a) water; b) ethanol 40%; c) ethanol 70%; d) ethanol 96%.

Table 2. The content of carotenoids and flavonoids in carrot pulp

| A variety of carrots from which pulp is obtained | The content of carotenoids, mg β-carotene in 100 g of dry weight | Flavonoid content, mg catechin equivalent in 100 g dry weight |
|-------------------------------------------------|---------------------------------------------------------------|----------------------------------------------------------|
| Losinoostrovskaya                               | 23.56±0.23                                                   | 12.02±0.37                                               |
| Nantes (Nantskaya)                             | 25.32±0.18                                                   | 13.45±0.56                                               |
| Queen of Autumn (Koroleva Oseni)               | 20.78±0.25                                                   | 11.50±0.48                                               |

The content of bioactive substances in vegetables is highly dependent on the variety and yield. Also, the production technology will affect the content of substances in the waste of industrial processing of carrots. Given these factors, the obtained values of the content of substances in general are consistent with the available data [16]. The content of carotenoids in pulp is generally lower than the content in the feedstock. While the content of flavonoids in carrots is usually approximately equal to or higher than the content of carotenoids, the content of flavonoids in the studied pulp samples was slightly lower than that of carotenoids. It can be explained by the uneven transition of substances into juice during technological processing. It can also be explained as finding a part of bioactive substances in vegetables in a bound state [14].

5. Conclusion

The article studied the content of the main groups of bioactive substances in the pulp obtained during the industrial processing of carrots. No high levels of carotenoids and flavonoids were detected, as well as no predominant number of carotenoids. It is assumed that in order to achieve the necessary efficiency in the isolation of bioactive compounds from carrot pulp, the development of new technological methods is necessary. One of the directions in the development of such methods can be methods for increasing the yield of bound phenolic compounds with high biological activity.
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