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Development of a method for isolating carotenoids from tomato mass

Absract. The article discusses a method for isolating carotenoids from tomato paste unsuitable for use in the food industry. It is known that the content of lycopene in tomatoes of various varieties varies in wide ranges, therefore, to obtain a natural preparation of lycopene, it is necessary to select varieties of tomatoes with its high content. Carotenoids are practically the only and relatively affordable source of vitamin A (retinol), and in most cases, their use does not lead to an overdose of this vitamin. It should also be noted that despite the wide range of biological activity of carotenoids and the fact that the substances of this class are non-toxic, readily available and relatively cheap to obtain, only some foreign medicines based on β-carotene are known (usually in combination with vitamins E and C). However, the development of effective means of drug therapy based on this class of compounds requires a detailed comparative study of their biological activity. Currently, carotenoids such as beta-carotene, lycopene, lutein, and astaxanthin have been widely used as biologically active components. This is due to their antioxidant, immunostimulating, anticarcinogenic and other features. The difficulty of isolating carotenoids is that representatives of this class in the plant world are in the form of associates with various biopolymers. One of the important organic compounds of natural origin is a fat-soluble carotenoid – lycopene. It is a tetraterpene, which consists of eight isoprene units, a valuable food coloring and antioxidant. The special value of lycopene is that, being a strong antioxidant, it effectively helps to reduce the concentration of harmful cholesterol in the blood. Taking lycopene, a person enhances his defense related to the violation of acid-base balance in the body, as well as problems of the prostate and potency. Also, according to several studies, lycopene can slow down the aging process. We have developed a method for the isolation of lycopene and β-carotene from the tomato mass, selected the optimal extraction conditions. Of all the tested solvents, the optimal extractants were: methyl chloride, acetone and chloroform, since the carotenoids are most completely extracted in these solvents. The extraction process, the amount of carotenoids from the tomato mass was carried out by hot maceration. Also, methods of standardization of isolated substances have been developed; UV-spectrometry has been selected as the most convenient and sufficiently accurate method.
Key words: tomato masses, carotenoids, lycopene, extraction, UV spectrometry

Introduction

Currently, it is well known that reactive oxygen species and free radicals are actively involved in the pathogenesis of many diseases. The flow of free radical reactions in the lipid phase of biomembranes leads to a violation of their physicochemical properties and changes in the operation of membrane enzyme systems.

Under normal conditions, aerobic organisms are protected from such effects by the coordinated functioning of various antioxidant systems, as well as a certain correspondence between the rates of metabolic processes and catabolism occurring in the cell. However, during shifts in the stationary course of radical processes, the modulation of the antioxidant status of the organism is required. In this regard, a great interest has recently manifested itself in carotenoids – natural pigments synthesized by plants and microorganisms.

Carotenoids are organic compounds made up of eight isoprene fragments. The activity of carotenoids, as antioxidants, is associated with the presence of a functional polyene chain in them. The main function of carotenoids in the plant cell is to protect its structures from the damaging effects of free radicals formed during photosynthesis. In animals, carotenoids are not synthesized in vivo; however, when

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ingested with a plant diet and involved in cell metabolism, they exhibit a certain biological activity. In addition to the provitamin function recently detected in P-carotene and other carotenoids, antioxidant activity helps to explain their role in preventing the development of cataracts, radiation damage, the occurrence and development of cardiovascular diseases, and in inhibiting mutagenesis and transformation of eukaryotic cells [1].

Carotenoids are practically the only and relatively affordable source of vitamin A (retinol), and in most cases their use does not lead to an overdose of this vitamin, because the synthesis of retinol during the metabolism of carotenoids is enzymatically limited [2].

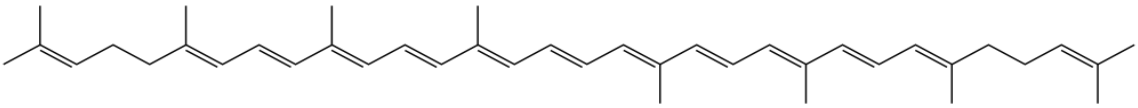
It should also be noted that despite the wide range of biological activity of carotenoids and the fact that the substances of this class are non-toxic, readily available and relatively cheap to obtain, only some foreign medicines based on β-carotene are known (usually in combination with vitamins E and C).

In the Republic of Kazakhstan, drugs, both on the basis of β-carotene, the most popular representative of the carotenoid class, and on the basis of its structural analogues, do not exist. Along with this, it is known that other carotenoids similar in structure to β-carotene, such as astaxanthin, canthaxanthin and lycopene, are stronger antioxidants [1].

However, the development of effective means of drug therapy based on this class of compounds requires a detailed comparative study of their biological activity.

Currently, carotenoids such as beta-carotene, lycopene, lutein, and astaxanthin have been widely used as biologically active components. This is due to their antioxidant, immunostimulating, anticarcinogenic and other features.

The body's need for a rational and balanced diet increases dramatically. An important role in the regulation of metabolic processes is played by such components of foodstuffs as biologically active lipids, and, in particular, carotenoids. Existing methods for the isolation of drugs – the amount of carotenoids from plant materials are few. The difficulty of isolating carotenoids is that representatives of this class in the plant world are in the form of associates with various biopolymers. One of the important organic compounds of natural origin is a fat-soluble carotenoid – lycopene. It is a tetraterpene, which consists of eight isoprene units, a valuable food coloring and antioxidant. The presence of 11 conjugated double bonds causes the light-absorbing property of lycopene and its ability to easily oxidize, therefore, during the oxidation of lycopene, epoxides of different composition are formed. Lycopene absorbs all wavelengths of visible light, so it has a red color [3].



Lycopene

The special value of lycopene is that, being a strong antioxidant, it effectively helps to reduce the concentration of harmful cholesterol in the blood. Taking lycopene, a person enhances his defense related to the violation of acid-base balance in the body, as well as problems of the prostate and potency. Also, according to several studies, lycopene can slow down the aging process. The main function of lycopene in the human body is antioxidant. Reducing oxidative stress slows down the development of atherosclerosis, and also provides DNA protection that can prevent oncogenesis. The consumption of lycopene, as well as lycopene-containing products leads to a significant decrease in markers of oxidative stress in humans. Lycopene is the most powerful antioxidant carotenoid present in human blood. Several pilot studies suggest a signaling role for lycopene in relation to certain cell cultures. In particular, it is assumed that lycopene can slow down cell proliferation as a signal metabolite [3-7].

Lycopene – refers to carotenoids and is synthesized by plants, algae and fungi. It has a thick pink color, turning into a purple hue, which is of great interest for the food industry, which is in dire need of natural harmless dyes of this hue. The use of lycopene for imparting pink color to food products provides them with an improved presentation and allows you to simultaneously enrich the products with valuable biological properties that contribute to the preservation of health [8-9].

Materials and methods

Tomato mass has a dark red color, with a specific smell.

The object of study is tomato paste with expired shelf life, prepared according to the following procedure:

Tomato paste was dried at room temperature for 10 days;

the resulting product has a burgundy color. Figure 1 shows the object of study.

Figure 1 – Dried Tomato Mass

Selection of conditions for the isolation of carotenoids: To isolate carotenoids from the tomato mass, work was carried out to select the optimal extractant.

For the extraction process we have chosen the following solvents: Acetone, hexane, ethanol, chloroform and methylene chloride

The extracts were prepared according to the following procedure: A tomato (exact weight) mass and extractant were placed in a 25 ml flask in a ratio of 1:5, then the flasks were closed and placed in a cabinet without sunshine for 24 hours.

The extract obtained after infusion must be filtered from the insoluble fraction.

Then the obtained extracts need to be placed in porcelain cups brought to constant weight and the extractant is completely evaporated, then, based on the weight of the dry residue, to calculate the proportion of extractives.

In parallel, according to a similar method, samples are prepared for qualitative and quantitative analysis by spectrometry and thin layer chromatography.

Carotenoid separation and lycopene detection in the proposed extracts: To this end, we have carried out work on the selection of systems for dividing the sum of carotenoids. The following solvents were used to prepare the systems: chloroform, hexane, acetone, ethyl, butyl alcohol, methyl chloride and acetic acid.

The deceleration rate (Rf) is determined - a characteristic of the relative speed of movement of a component in a thin layer (also known as retention coefficient (Rf) in planar chromatography). The deceleration factor is equal to the ratio of the distance from the sample application point to the center of the adsorption zone and the distance traveled by the solvent front from the sample application point. For chromatography, we used the solvent systems hexane - acetone and hexane - chloroform.

In addition, a quantitative analysis of carotenoids and lycopene was performed by UV spectrophotometry by the following method:

Approximately 1 g (exact mass) of the drug is dissolved in a mixture of these solvents in a 50 ml volumetric flask, diluted to the mark with the same mixture and stirred.

The optical density of the solution is measured at a wavelength of 450 nm in a cuvette with a layer thickness of 10 mm, using as a reference solution a mixture of hexane-ethyl alcohol 96%.

In parallel, measure the optical density of the solution with potassium dichromate.

Results and discussion

The results of the study of the extract content of substances from the tomato mass and data on the qualitative composition of carotenoids are presented in table 1 and in figure 2.

Table 1 – The effect of solvents on the quality of the extraction of carotenoids in tomato mass

Solvents	Indicators			
	The amount of extractives , %	Carotenoids	Lycopene	Terpenes and other classes of compounds
Acetone	33,15	+ +	+ +	+ + +
Ethanol	34,20	+	+	++ +
Hexane	30,35	+	+	++ +

Continuation of table 1

Solvents	Indicators			
	The amount of extractives , %	Carotenoids	Lycopene	Terpenes and other classes of compounds
Chloroform	34,45	++	++	+++
Methyl chloride	38,25	+ + +	+ ++	+ ++
Note: "+" intensity of spots on a thin-layer chromatogram				

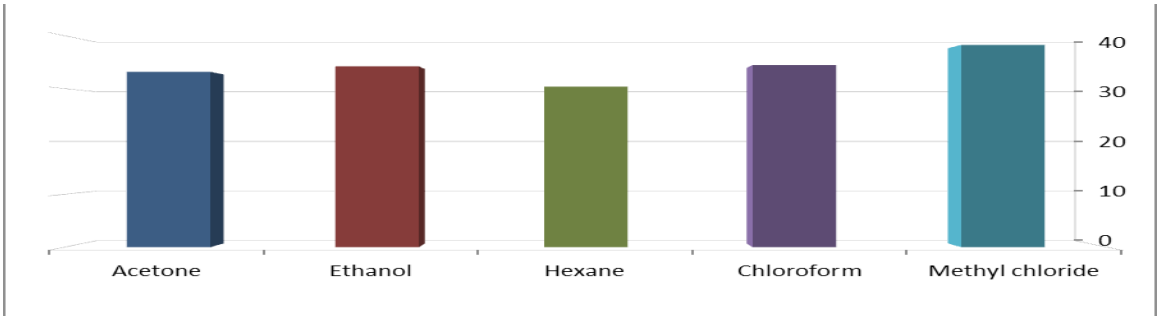


Figure 1 – The yield of extractives from raw materials, depending on solvents

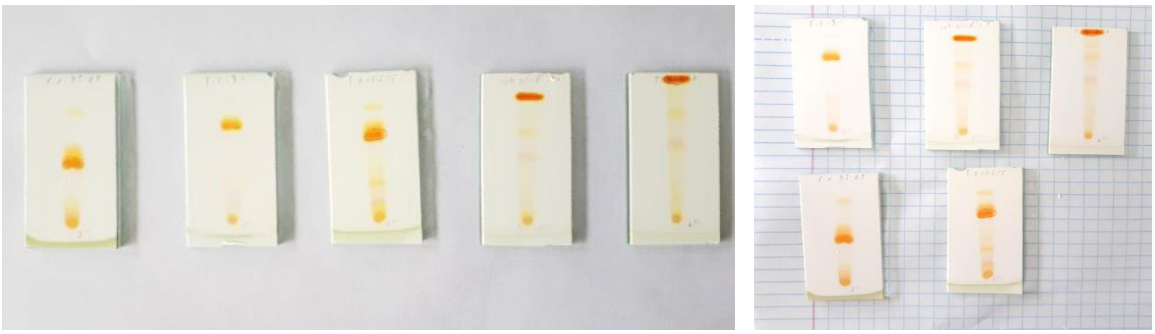


Figure 2 – Analysis of carotenoid concentrate from tomato mass by thin layer chromatography

Of all the tested solvents, the optimal extractants were: methyl chloride, acetone and chloroform, since the carotenoids are most completely extracted in these solvents.

The extraction process, the amount of carotenoids from the tomato mass was carried out by hot maceration.

The basic flow chart of the production of the amount of carotenoids from tomato includes the following technological processes (TP):

TP.1 – harvesting plant materials;

TP.2 – drying of vegetable raw materials in a ventilated room at a temperature of 200 ° C without direct sunlight;

TP.3 – control of the good quality of crushed vegetable raw materials;

TP.4 – grinding of plant materials at the mill-carved type SM 100 comfort with a 5 liter receiver, rotor and filter bag to a particle size of 1-2 mm;

TP.5 – supercritical fluid extraction using a Thar SFE-1000 instrument;

TP.6 – removal of ballast substances;

TP.7 – final purification (chromatographic analysis) and isolation of the final product.

The extract obtained is concentrated and subjected to chromatography on a thin layer of silica gel.

Carotenoid separation and lycopene detection in the proposed extracts. To this end, we have carried out work on the selection of systems for dividing the sum of carotenoids. The following solvents were used to prepare the systems: chloroform, hexane, acetone, ethyl, butyl alcohol, methyl chloride and acetic acid.

The deceleration rate (Rf) is defined as a characteristic of the relative speed of movement of a component in a thin layer (also known as retention coefficient (Rf) in planar chromatography). The deceleration factor is equal to the ratio of the distance

from the sample application point to the center of the adsorption zone and the distance traveled by the solvent front from the sample application point.

Currently, we have studied about 40 systems, of which the best were: hexane – acetone (9: 1), hexane – chloroform (9: 1) and it was established that in the system hexane – acetone (10: 0.5) lycopene has Rf = 0.62.

In addition, a quantitative analysis of carotenoids and lycopene was performed by UV spectrophotometry.

The research results are summarized in table 2.

Table 2 – The quantitative content of carotenoids in tomato mass (mg%)

λ, nm	β-carotene	Lycopene
474	-	13,30
474	-	13,37
474	-	13,36
450	15,14	-
450	14,86	-
450	14,88	-

It was established that at λ = 474 nm, the content of lycopene is 13.34 mg%, and the content of β-carotene at λ = 450 nm was 14.96 mg%.

Conclusions

– Thus, the tomato mass, obtained from tomato paste, which has expired, dried under a plaster, at room temperature for 10 days. Tomato mass has a dark red color, a specific smell.

– Tomato pulp was treated with hexane, ethyl alcohol, acetone – ethyl alcohol mixture at a ratio of (5: 1) and methyl chloride. The most saturated extract is obtained by methyl chloride. The number of extractives in the studied solvents.

– Using the TLC method, a system was selected where it is possible to qualitatively determine the presence of lycopene and other carotenoids in the plant object.

– Quantitative analysis of carotenoids and lycopene was determined by UV spectrophotometry.

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