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DEVELOPMENT OF THE RETRIEVING TECHNOLOGY OF CAROTENOIDS FROM PUMPKIN (CUCURBITA SPP.) PULP USING Zn-Al LAYERED DOUBLE HYDROXIDES

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Carotenoids are biologically active materials with strong antioxidant properties, some of them are provitamins A. A promising source of carotenoids is pumpkin pulp. The object of research is the technology of retrieving carotenoids using LDH.

The flowsheet for obtaining pumpkin carotenoids by precipitation of the carotenoids-LDH composite was developed:

a) obtaining fresh pumpkin juice with the introduction of Zn and Al salts;

b) precipitating the carotenoids-LDH composite by adding alkali to pH=9 at t=60 °C and stirring;

c) filtering the precipitate of the composite under vacuum, drying, rinsing, re-filtering, and re-drying;

d) separating the composite into components.

A simple mechanical method (grinding and sieving) was proposed to separate the composite into carotenoid-enriched and LDH-enriched materials. The method is based on the internal self-abrasion of the composite when grinding solid particles of LDH as grinding bodies. When removing carotenoids in the form of a composite, rapid precipitation of the sediment and ease of filtration under vacuum were found. X-ray diffraction analysis showed that the composite and products of its separation contain X-ray amorphous Zn-Al LDH, an oxide phase, and an amorphous phase of carotenoids. The method of dichloroethane extraction proved the effectiveness of the composite separation process. It was shown that for the optimal amount of Zn-Al LDH, the content of carotenoids in carotenoid-enriched material was 24.4 %, and in LDH-enriched – 4.4 %. For these conditions, it was found that the total yield of carotenoids was 184.3 mg/100 g of pumpkin pulp, of which 155.4 mg/100 g was in the carotenoid-enriched material and 28.9 mg/100 g was in the LDH-enriched material. A hypothesis was expressed regarding the chemical nature of the interaction of carotenoids and LDH in the composite due to π-d interaction.

The resulting carotenoid-containing materials can be used as food additives or processed to obtain purified carotenoids

Keywords: Zn-Al layered double hydroxide, carotenoids-layered double hydroxide composite, carotenoid retrieving technology, pumpkin pulp, internal self-abrasion

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1. Introduction

Carotenoids are natural organic pigments that are synthesized by plants, bacteria, fungi, and algae. These pigments give plants, vegetables, and fruits a bright yellow, red, and orange color. Among over 600 known types of carotenoids, the most common are α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene [1]. Carotenoids are divided into two classes: xanthophylls and carotenes. Xanthophylls contain oxygen in their structure and have a more yellow tint. This class includes lutein and zeaxanthin. Carotenes do not contain oxygen and tend to be associated with a more orange pigment color. This class includes α-carotene, β-carotene, β-cryptoxanthin, and lycopene. All carotenoids are biologically active substances. α-carotene, β-carotene, and β-cryptoxanthin are provitamins that are converted into vitamin A in the body. Other carotenoids are not vitamins but are strong antioxidants [2]. Carotenoids have a broad commercial application [3] as both biologically active and food additives. As food additives, carotenoids are used as antioxidants and natural pigments [4, 5].

Despite the developed methods of synthesis, carotenoids are obtained from natural sources, mainly cultivars. The most commonly used microorganisms to obtain carotenoids are microalgae [6], yeast, *Escherichia coli* bacteria [7], and *Lactobacteria plantarum* [8]. The main source of lycopene is tomatoes [9], and the main source of lutein is the petals of marigolds *(Tagetes erecta)*. Carotenoids are found in various fruits and vegetables [10–12]. In Ukraine, as well as in the countries of Eastern Europe, the Middle East, and Latin America, a promising source of carotenoids (carotenes, lutein, and zeaxanthin) are pumpkin fruits. In particular, a special selection of pumpkin varieties with a high content of carotenoids is being carried out. The development of new technologies for retrieving carotenoids from pumpkin pulp is a promising direction. The introduction of methods for obtaining carotenoids without using harmful or flammable materials will improve industrial technology.

2. Literature review and problem statement

The main method for retrieving fat-soluble carotenoids from plant materials is liquid extraction with organic solvents. In [13], ultrasound was used to improve the extraction of lycopene from tomatoes. Lycopene can be obtained from tomato waste [14], at the same time, to improve the extraction, the work [15] suggests using enzymes, and [16] – pre-drying of the cake. In [17], it is proposed to use a solvent obtained from orange waste as a “green” extractant. The main disadvantages of organic solvent extraction are the need to use large volumes of flammable liquid, the need for its regeneration, and a rather high residual solvent content. These shortcomings can be avoided by using supercritical CO_2 as an extractant in the extraction of β-carotene [18] and lycopene [19]. However, the application of CO_2 supercritical fluid requires the use of complex equipment, which is badly scaled.

In [20], a method for extracting lycopene using layered double hydroxides (LDH) was proposed, and the high antioxidant activity of the obtained materials was revealed [21]. Lycopene retrieving was carried out in the form of a carotenoid nanocomposite with Zn-Al LDH, the structure of which is similar to classical composites [22], and especially to metal-ceramic composites [23].

Hydroxides of divalent metals (except for alkaline earth metals) are characterized by polymorphism and two modifications were described [24]. The β-modification is described by the chemical formula $Me(OH)_2$ and has the structure of brucite. β-hydroxides of electrochemically active metals (for example, Ni) are used in chemical current sources, including supercapacitors [25]. The α-modification (chemical formula $3Me(OH)_2 \cdot 2H_2O$, hydrotalcite structure) has a higher chemical and electrochemical activity and is widely used, for example, in electrochromic devices [26, 27]. At the same time, structures intermediate between the α- and β-forms were described for nickel hydroxide [24]. The works [28, 29] described the formation of $Ni(OH)_2$ with a mixed layered (α+β) structure characterized by increased activity [30].

Layered double hydroxide (LDH) is an α-modification of the host metal hydroxide, in the crystal lattice of which some of the host metal cations are replaced by guest metal cations: for example, Ni^{2+} or Zn^{2+} (a host) is replaced by Al^{3+} (a guest) [31, 32]. As a result, an excess positive charge is formed in the crystal lattice, which can be compensated by the intercalation of additional anions into the interlayer space. Such anions can be counterions of precursor salts [33, 34]. But most often, anions with special functional properties are purposefully intercalated into LDH structures. Stabilizing [35, 36] or activating anions [37], increasing the electrochemical activity, can be introduced into the LDH composition.

Thus, the LDH structure is formed by the following main components [38]: host metal cations, guest metal cations, and intercalated anions. LDH with the required characteristics can be created using ion design, with a targeted choice of these three components.

Intercalation of LDHs with anionic dyes is widely used to obtain pigments. In this case, the pigment color can be determined by the color of the host and guest metal cations [38, 39], the color of the intercalated anion [40, 41], and a combination of both colors [42]. For the synthesis of pigments, intercalation with azo dye anions is used [43, 44]. In particular, Acid Yellow 17 was intercalated in Zn-Al LDH [45], o-Methylene Red was intercalated in Mg-Al LDH [46] and Ni-Fe LDH [47], Mordant Yellow 3 was intercalated in Zn-Al LDH [48, 49], Acid Yellow 3 in Zn-Al [50], Acid Green 28 in Zn-Al [51].

A promising direction is the use of LDH as a nanocontainer for special functional anions [52] of organic [53] and inorganic [54] nature, for example, anions for sensors [55] and corrosion inhibitors [56]. LDHs can also be successfully used to obtain cosmetic [57, 58] and medical materials [59]. Thus, medicinal antitumor [60] and anticancer agents [61], and tryptophan-intercalated LDH [62] can be synthesized. Taste [63, 64] and aromatic [65] food additives and cosmetic pigments [39, 40] can also be obtained by intercalation, including intercalation of natural dyes from spices [66]. In [66], water-soluble carotenoids (crocin, crocetin) from an aqueous saffron infusion were intercalated in Zn-Al LDH.

It should be noted that all the described materials obtained using LDH are the result of the intercalation of water-soluble anionic substances. At the same time, the main pumpkin carotenoids (β- and α-carotenes, β-cryptoxanthin, lycopene, lutein, and zeaxanthin) are fat-soluble, insoluble in water, and have a non-ionic structure. Therefore, the retrieving of carotenoids by intercalation in LDH is impossible. However, it is known that carotenoids form rather large formations in the fruit pulp, which can be separated from the fibers, peel, and seeds. But further filtering of the obtained juice with pulp is very difficult. Therefore, the authors of [20] proposed a method for separating lycopene from the liquid of fresh tomato juice by coagulation with Zn-Al LDH to form a nanocomposite. At the same time, the composite was quite easily separated from the mother liquor and had high thermal stability, which made it easy to filter the precipitate and dry it from water without vacuum and at elevated temperatures. This method is promising and can be taken as the basis for the developed technology for retrieving carotenoids from pumpkin pulp.

The main unresolved problem is the transformation of the method of obtaining carotenoids using LDH into a technology. The method for obtaining carotenoids proposed in [20] cannot be taken as technology, since it has several significant drawbacks. The method was not tested for other natural sources of carotenoids, in particular pumpkin. The studies were carried out on very small volumes of fresh juice, which does not allow predicting the possibility of scaling. Filtration of the lycopene-LDH nanocomposite precipitate was carried out only after centrifugation, which significantly complicated the process. At the same time, the authors did not even suggest the possibility of a simple separation of the composite either into pure components (lycopene and LDH) or into two phases: the 1st is predominantly carotenoid and the 2nd is LDH. They have an admixture of another component as well.

3. The aim and objectives of the study

The work aims to develop a technology for retrieving carotenoids from pumpkin pulp using layered double hydroxides, with the development of individual elements of the technology. This will make it possible to obtain carotenoids or concentrated carotenoid-containing materials from a promising natural source – pumpkin pulp.

To achieve the aim, the following objectives were set:

– to develop a general flowsheet for obtaining carotenoids from pumpkin pulp by precipitation of the carotenoids-LDH composite;

– to propose a method for separating the precipitated composite into carotenoid and LDH components (or components with a maximum carotenoid content and a maximum LDH content);

– to carry out the retrieving of carotenoids from fresh pumpkin pulp juice to obtain a carotenoids-LDH composite, at different amounts of LDH, to investigate the phase composition of the composite and products of its separation;

– to investigate the content (wt.%) and yield of carotenoids (mg/100 g of pumpkin pulp) in the separation products of the carotenoids-LDH composite by the extraction method for evaluating the efficiency of the separation method and determining the optimal amount of LDH.

4. Materials and methods

4. 1. Object and hypothesis of the study

The object of the study is the technology of extracting carotenoids using LDH. Two hypotheses were used. The first hypothesis is that during the LDH precipitation in the medium of pumpkin pulp juice, a precipitate of the carotenoids-LDH composite will be formed. The second hypothesis is the possibility of mechanical separation of the composite due to internal self-abrasion.

The main assumption is that only carotenoids are precipitated together with LDH during the formation of the composite.

4. 2. The principle of developing a flowsheet for obtaining carotenoids from pumpkin pulp

The flowsheet should include a sequence of processing stages, starting from the feedstock (pumpkin pulp) and ending with the production of carotenoids and LDHs (carotenoids- or LDH-enriched materials). Each stage of the flowsheet should be, if possible, with simple instrumentation.

4. 3. The principle of developing a method for the separation of the carotenoids-layered double hydroxide composite

The method for separating the carotenoids-LDH composite into components should be simple, not require sophisticated equipment and the use of toxic, expensive, and flammable substances. At the same time, the method should be easily scalable.

When developing the separation method, it was decided to abandon the separation by organic solvent extraction due to significant drawbacks, the main of which is the combustibility of organic extractants.

It is shown in [20] that the carotenoid-LDH composite, obtained by the formation directly in the fresh juice medium, consists of small-sized LDH particles and a carotenoid. The components of the composite have very different properties. In particular, carotenoids, as organic non-ionic substances, have very low hardness, while LDHs, as ionic substances of a hydroxide-salt nature, have very high hardness. Based on this, a hypothesis was put forward that during the grinding of the composite, when minor mechanical forces are applied to the particles, the self-abrasion of the composite will occur. In this case, the solid LDH particles will play the role of internal grinding bodies, abrading the carotenoid phase. After mechanical grinding, the formation of particles of two different materials should occur – small particles of carotenoids and larger particles of LDH-enriched material, which are LDH particles coated with a residual layer of carotenoids. These materials can be separated by sifting using a 50–70 μm sieve.

4. 4. Method for extracting carotenoids from fresh pumpkin pulp juice

To obtain the carotenoids-LDH composite, samples of large-fruited pumpkin Cucurbita maxima were used, with the peel, seeds, and placenta removed. For each experiment, 200 g of pumpkin pulp was taken. The pulp was crushed with a blender, filled with a double volume of distilled water, and thoroughly mixed. After that, for the preparation of fresh juice, the fibers and the cake were separated by vacuum filtration three times through a Ni mesh with a successively decreasing mesh size (minimum size of 200 μm). Each time after filtration, the precipitate was rinsed with a stream of distilled water.

To extract carotenoids, preliminarily calculated amounts of crystalline hydrates of zinc and aluminum nitrates were introduced into the obtained fresh pumpkin juice. Five versions of the experiment were carried out with different amounts of the LDH formed (at a molar ratio of $Zn^{2+}:Al^{3+}=3:1$). The calculation of the amount of zinc and aluminum salts for the basic option (option **C**) was carried out based on the formation of ascorbate-intercalated Zn-Al LDH, taking into account the average content of ascorbic acid in pumpkin pulp. For option **B**, the amount of LDH was reduced by 25 %, for option **A**, the amount of LDH was reduced by 50 %, for option **D**, the amount of LDH was increased by 25 %, and for option **E**, the amount of LDH was increased by 50 %.

When retrieving carotenoids by forming the carotenoids-LDH composite, a KOH solution was added to fresh pumpkin juice with dissolved zinc and aluminum nitrates at a temperature of 60 °C with continuous stirring until pH=9 was reached. Stirring was continued for 30 minutes after the completion of the composite formation process. The formed precipitate of the composite was filtered from the mother liquor under vacuum and dried for 24 hours at 60 °C, soaked in distilled water, filtered, and dried again under the same conditions. Before investigating the characteristics, the composite samples were divided into two phases: carotenoid-enriched (labeled **ZnAl-P-Carotenoid**) and LDH-enriched (labeled **ZnAl-P-LDH**). Full sample labeling also includes the synthesis option, e.g. **ZnAl-P-Carotenoid-C**.

4. 5. Methods for investigating the characteristics of the obtained samples of carotenoid-containing materials

Investigation of structural characteristics.

The crystal structure of the materials was studied using X-ray diffraction analysis (XRD) on a DRON-3 diffractometer (Co-Kα radiation, angle range 10–90° 2θ, scanning rate 0.1°/s).

Investigation of the carotenoid content in the obtained materials and the carotenoid yield (mg/100 g of pumpkin pulp).

An extraction-gravimetric method was used to determine the content of fat-soluble carotenoids in the pumpkin. Its essence lies in the fact that 0.1 g of the material (carotenoid-enriched or LDH-enriched) was poured into 25 ml of 1,2-dichloroethane and the extraction was carried out in a closed air-tight glass weighing bottle for 24 hours at room temperature. The resulting extract with a solid residue was transferred to a pre-weighed paper filter (pore diameter 1–2 μm), on which the gravitational separation of the filtrate into a pre-weighed Petri dish took place. The solvent (1,2-dichloroethane) was evaporated to dryness from the Petri dish, the weighing bottle, and the paper filter, followed by weighing. Solvent evaporation was carried out in two versions: at room temperature and at 60 °C. After weighing, the balance of the obtained material was compiled, and the weight gain of the Petri dish was taken as the mass of carotenoid in the sample. After that, the content (wt. %) and yield of carotenoids (mg/100 g of pumpkin pulp) were calculated for all samples obtained.

5. Results of the development of technology for retrieving carotenoids from pumpkin using Zn–Al layered double hydroxide

5. 1. Results of the development of a flowsheet for obtaining carotenoids from pumpkin pulp

Based on the characteristics of the starting materials and products, the following flowsheet was proposed:

First stage. Making fresh juice from pumpkin pulp. At the first stage, it is necessary to thoroughly grind the pumpkin pulp, add deionized water, and perform multi-stage filtration (3–4 stages) from fibers and cake through solid filters with decreasing pore size. Filtration must be carried out under a vacuum, with the possibility of rinsing with deionized water. After drying, the resulting cake can be sent for the extraction of the remaining carotenoids or for other use.

Second stage. Obtaining the carotenoids-LDH composite. It must be carried out in a jacketed reactor to maintain temperature, with continuous stirring and pH control (in operando). Before obtaining, the required amounts of zinc and aluminum nitrates are added to fresh juice and mixed until dissolved. After that, it is necessary to add gradually an alkali solution to fresh juice to the required pH. After reaching the required pH, the suspension must be kept at the temperature of preparation and continuously stirred for 30–60 minutes. The amount of salts introduced, the rate of alkali addition, the final pH value upon receipt of the composite, and the temperature must be optimized according to the results of experimental studies.

Third stage. Filtration and drying of the composite. The resulting precipitate of the composite must be filtered under vacuum (using a filter press or vacuum filter) and dried at a temperature of 55–65 °C. After drying, the composite must be soaked in deionized water, re-filtered, and dried at the same temperature.

Fourth stage. Separation of the composite. It is necessary to develop a simple method for separating the composite into two components. An ideal option is the separation into a mixture of carotenoids and LDHs, which will be the products of the technology. However, the final products of the technology are more likely to be carotenoid-enriched and LDH-enriched materials.

5. 2. Results of the development of the method for separating the precipitated composite into components

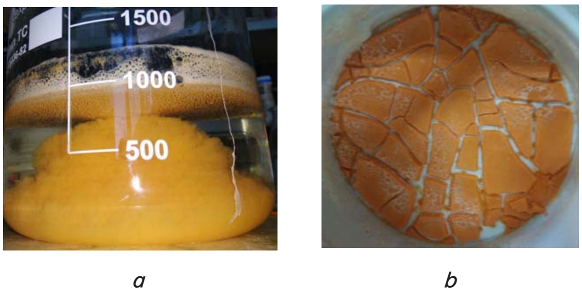
To test the hypothesis about the possibility of mechanical separation of the composite, a sample of the **ZnAl-P-Composite-C** composite (basic precipitation option) was manually ground (Fig. 1, *a*) in a ceramic mortar with a ceramic pestle without significant effort for 10 minutes. The ground composite was sifted through a 50 μm Ni mesh. The material sifted through the sieve was finely dispersed, very soft, and yellow-orange in color (Fig. 1, *b*). This material is most likely to be carotenoid-enriched.

Fig. 1. Photo images of materials obtained by mechanical separation (grinding-sifting) of the **ZnAl-P-Composite-C** composite sample: *a* – initial composite; *b* – **ZnAl-P-Carotenoid-C** sample; *c* – **ZnAl-P-LDH-C** sample

The residue on the sieve was coarse, contained solid particles, and had a dark orange color (Fig. 1, *c*).

5. 3. Results of obtaining the composite with different amounts of layered double hydroxide and investigating the phase composition

Adjusting the pH of fresh pumpkin juice with dissolved zinc and aluminum nitrates to 9 at any amount of salts led to the formation of a precipitate of the carotenoids-LDH composite and its rapid coagulation (Fig. 2, *a*). The precipitate was easily filtered out under vacuum (Fig. 2, *b*).



a *b*

Fig. 2. Photo images of the **ZnAl-P-Composite-C** composite sample: *a* – in the mother liquor during coagulation (after 10 min.); *b* – after vacuum filtration

The crystal structure of the **ZnAl-P-Composite-C** composite sample, as well as the products of its separation **ZnAl-P-Carotenoid-C** and **ZnAl-P-LDH-C**, was studied by XRD (Fig. 3).

The diffraction pattern of the initial **ZnAl-P-Composite-C** composite shows peaks in the crystal lattice of α-$Zn(OH)_2$, which corresponds to Zn-Al LDH, for example, at $2\Theta=14^\circ$. The LDH peaks have a small height and a large width, which indicates that the LDH is X-ray amorphic. A halo of the amorphous component is observed in the region of the LDH peaks. The diffraction pattern has all three clear characteristic peaks of zinc oxide at $2\Theta=37.4^\circ$, 40.6°, and 42.8°, which indicates the presence of ZnO in the composite.

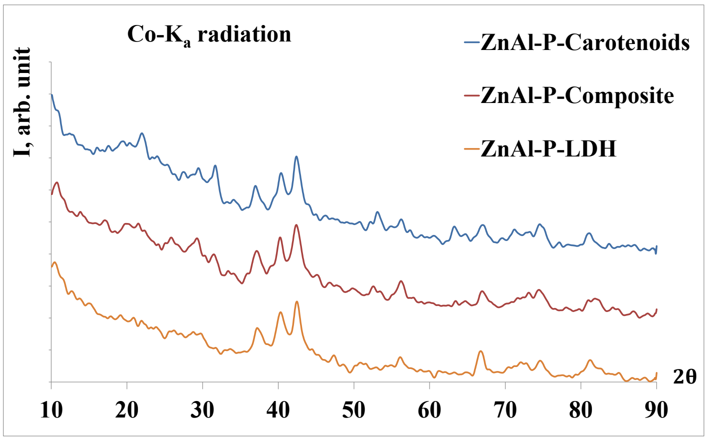


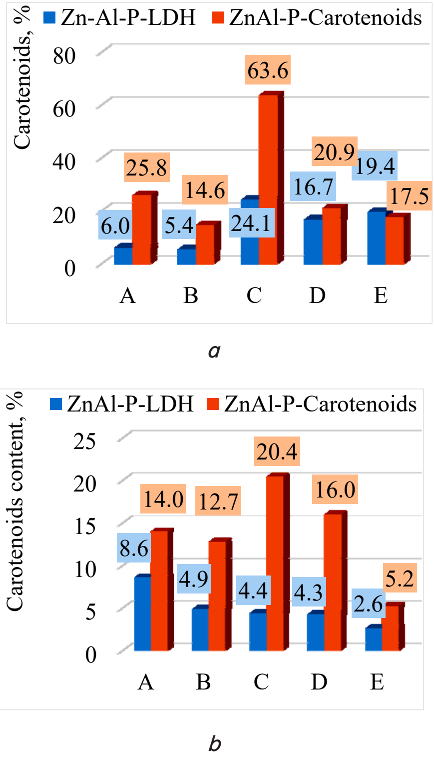
Fig. 3. Results of XRD analysis of the ZnAl-P-Composite-C composite sample and products of its mechanical separation ZnAl-P-Carotenoid-C and ZnAl-P-LDH-C

The diffraction patterns of the initial **ZnAl-P-Composite-C** composite and **ZnAl-P-LDH-C** and **ZnAl-P-Carotenoid-C** materials are almost identical. In the region of small angles $2\Theta\leq10^\circ$, a significant halo of amorphous material is observed, which most likely corresponds to carotenoids.

5. 4. Results of the investigation of the content and yield of carotenoids

The results of determining the content of carotenoids in carotenoid- and LDH-enriched samples are shown in Fig. 4, *a*, and the yield of carotenoids is shown in Fig. 4, *b*. The results of compiling the material balance show that during the evaporation of dichloroethane at room temperature, the solvent was not completely removed. Therefore, the data shown in Fig. 4 were obtained at an extractant evaporation temperature of 60 °C.

The carotenoid content, determined by extraction, confirmed the correctness of the mechanical method for separating the carotenoids-LDH composite. All samples named **-Carotenoids** contained significantly more carotenoids than the corresponding **-LDH** samples. The composite samples obtained with the basic option of the LDH amount (option **C**) had the best indicators: 20.4 % carotenoids in the **ZnAl-P-Carotenoid-C** sample and 4.3 % in the **ZnAl-P-LDH-C** sample. A decrease and increase in the LDH amount led to a decrease in the carotenoid content in the carotenoid-enriched samples, while the efficiency of phase separation in the composite decreased.



a

b

Fig. 4. Content and yield of carotenoids for production options with different amounts of layered double hydroxide: *a* – content of carotenoids in the samples, wt.%; *b* – yield of carotenoids in the samples, mg/100 g of pumpkin pulp

The carotenoid yield (mg/100 g of pumpkin pulp) was also maximum for the basic option **C** (Fig. 4, *b*), 155.4 mg/100 g for the **ZnAl-P-Carotenoid-C** sample and 28.9 mg/100 g for the **ZnAl-P-LDH-C** sample, the total yield of carotenoids was 184.3 mg/100 g.

6. Discussion of the results of the development of technology for retrieving carotenoids using Zn-Al layered double hydroxides

Development of technology for retrieving carotenoids from pumpkin pulp.

In the proposed flowsheet for carotenoids retrieving, the stage of preparing fresh pumpkin pulp juice is essential. The equipment for preparing pumpkin juice can be used for this. However, the technology should be modernized to maximize the conversion of carotenoids into fresh juice. It is also necessary to ensure the completeness of the separation of fibers and cake from fresh juice.

The technology of the stage of obtaining the carotenoids-LDH composite corresponds to the technology of the synthesis of dye-intercalated pigments [34, 41]. The completeness of coagulation of the composite precipitate is experimentally shown (Fig. 2, *a*). In contrast to the work [20], describing the use of centrifugation to accelerate the sedimentation of the lycopene-LDH composite in the mother liquor, in this investigation, the separation of the composite precipitate is effectively carried out using vacuum filtration. In this case, the filtration rate is high. Direct filtering has a significant advantage for the technology. At the same time, the layer of the composite precipitate on the filter in the Buchner funnel became covered with cracks at the end of vacuum filtration (Fig. 2, *b*). This is characteristic of hydroxide and LDH precipitates [29, 38, 41] and indicates reduced plasticity and brittleness of the precipitate.

Development of the method for separating the carotenoids-LDH composite.

In this study, a simple method for separating the components of the carotenoids-LDH composite was proposed, consisting of low-energy mechanical grinding and sifting through a 50 μm sieve. The method is based on the difference in the carotenoids and LDH hardness, and the possibility of internal self-abrasion of the composite. With low-energy grinding of the composite, the hard LDH particles will interact with each other and abrade the soft carotenoid to produce very fine particles. When sifting through a sieve, the material will pass through the labeling of which indicates **-Carotenoid**. The correctness of the proposed method is confirmed by the data shown in Fig. 4, *a*. The analysis of these data shows that the **ZnAl-P-Carotenoid-C** sample contains significantly more carotenoids than the **ZnAl-P-LDH-C** sample. It should be noted that the finer **ZnAl-P-Carotenoid-C** sample is actually a carotenoid-enriched material (carotenoid content of 20.4 %), while the **ZnAl-P-LDH-C** sample is LDH-enriched material (carotenoid content of 4.4 %). The X-ray diffraction analysis (Fig. 3) showed the presence of LDH and an oxide phase (probably Zn-Al layered double oxide (LDO)) both in the initial **ZnAl-P-Composite-C** composite and in both separation products, carotenoid-enriched **ZnAl-P-Carotenoid-C** and LDH-enriched **ZnAl-P-LDH-C**. The presence of LDH and LDO in the carotenoid-enriched phase can speak to the fact that during grinding in a mortar, not only internal self-abrasion can occur to obtain the carotenoid powder, but also partial destruction of LDH particles. Thus, it is most likely that the carotenoid-enriched sample is contaminated with mechanical impurities from the LDH-enriched sample. The presence of carotenoids in the LDH-enriched material is explained by the residual layer of carotenoids on the LDH particles.

Investigation of the content and yield of carotenoids.

In the investigation, the content and yield of carotenoids were determined for the samples obtained with various options of LDH amount during the composite formation. From the analysis of data, the content of carotenoids in the sample and the minimum content in the LDH-enriched sample are typical for the samples obtained by separating the composite obtained with the basic option of the LDH content (option **C**). The carotenoid separation coefficient (ratio of carotenoid content in carotenoid- and LDH-enriched materials) for option **C** was 4.64. With a decrease in the LDH content by 25 % (option **B**) and 50 % (option **A**), the separation coefficient decreases to 2.59 and 1.63, respectively. Such a decrease in the separation coefficient is probably associated with a decrease in the content of LDH solid particles as grinding bodies for self-abrasion. An increase in the LDH content by 25 % (option **D**) and 50 % (option **E**) also leads to a decrease in the separation coefficient to 3.72 and 2.0, respectively. The decrease in the separation coefficient is probably associated with an increase in the size of the LDH particles and the deterioration of their characteristics as grinding bodies for the internal self-abrasion of the composite. Thus, in the study of the carotenoid content in various samples, the possibility of separating the carotenoids-LDH composite by the mechanical method of internal self-abrasion and sieving was proved. In addition, it was found that in terms of the highest carotenoid separation coefficient (the best phase separation of the composite), the best option for the formation of the composite is the basic option of the LDH content, in which the amount of zinc and aluminum salts is calculated from the content of ascorbic acid.

Based on the results of dichloroethane extraction and gravimetry, the yield of carotenoids (mg/100 g of pumpkin pulp) for carotenoid- and LDH-enriched materials was calculated for all options of the LDH content (Fig. 4, *b*). The maximum yield was found for the basic option of the LDH content in the carotenoids-LDH composite (option **C**) and amounted to 155.4 mg/100 g of pumpkin pulp (**ZnAl-P-Carotenoid-C**) and 28.9 mg/100 g of pumpkin pulp (**ZnAl-P-LDH-C**), the total yield was 184.3 mg/100 g pumpkin pulp. The literature shows a very large scatter of data on the content of carotenoids in pumpkin pulp. It is indicated that the content of total carotenoids strongly depends on the biological species and variety of pumpkin, and the level of agricultural technology of cultivation. It is 11.2–15.47 mg/100 g for C. maxima varieties [67], 35 mg/100 g for C. maxima varieties [68], 15.49–18.40 mg/100 g [69], 17.0 mg/100 g for C. maxima varieties and 23.5 mg/100 g for C. moschata varieties [70], 8.62 mg/100 g for C. maxima varieties [71] and 4.43 mg/100 g for C. moschata varieties [72]. At the same time, some other sources give the carotenoid content of up to 250–300 mg/100 g. The obtained data on the yield of carotenoids generally correlate with the literature data but are still probably overestimated, given the non-optimal method for transferring carotenoids from pulp to fresh juice and a rather high residual content of carotenoids in the cake. This excess is probably due to non-selective extraction with 1,2-dichloroethane, which is able to dissolve not only carotenoids but also other water-insoluble substances that can pass from pulp to fresh juice and co-precipitate together with carotenoids into the forming composite. This fact is a drawback of the study, which can be eliminated using a more selective method of extraction and determination of carotenoids, such as liquid chromatography. An increase and decrease in the LDH content leads to a decrease in the yield of carotenoids: in the series of options **A** (-50 %) – **B** (-25 %) – **C** (basic option) – **C** (+25 %) – **D** (+50 %), the total yield of carotenoids (mg/100 g) was 63.9 – 82.9 – 184.3–80.0–20.9. This result is contradictory since all insoluble substances co-precipitate into the composite without any residue. However, during the extraction, it was observed that after the process of dissolution of carotenoids and other substances, the separated solid residue remained orange in color. It should be concluded that carotenoids from the surface of LDH particles are not dissolved after 24 hours of extraction. This indicates that carotenoids are chemically bonded to the LDH surface, probably due to the interaction of the system of conjugated π-bonds of carotenoids with Zn^{2+} d-orbitals in the LDH. When alkali is added to fresh pumpkin juice with dissolved Zn^{2+} and Al^{3+} salts, the LDH begins to form on the surface of carotenoid particles, as a result of which the carotenoid-LDH composite is formed. Probably, with a decrease and increase in the LDH content, the contact surface of carotenoid particles and LDH increases. With a decrease in the LDH content, the surface area increases due to the formation of submicron or nano-sized LDH particles. An increase in the LDH content also leads to an increase in the surface area due to an increase in the number of particles and the possible development of the surface. However, this explanation is a hypothesis that needs to be proved in future studies.

It should also be noted that the products of the proposed technology are carotenoid-enriched material and LDH-enriched material. These materials can be used directly, especially carotenoid-enriched one, given the very low toxicity of Zn-Al LDH [73], high antioxidant activity [21], and the described food additives in the form of a composite with Zn-Al LDH [74]. To implement the technology, further optimization of all stages is necessary. In addition, a possible option for the development of this study is the development of a technology for processing carotenoid-enriched material to obtain a purified mixture of carotenoids.

7. Conclusions

1. A general flowsheet for obtaining carotenoids from pumpkin pulp by precipitation of the carotenoids-LDH composite was developed. The flowsheet includes:

a) obtaining fresh pumpkin pulp juice with the introduction of the required amount of Zn and Al salts;

b) precipitating the carotenoids-LDH composite by adding alkali to pH=9 at a temperature of 60 °C and continuous stirring;

c) filtering the precipitate of the composite under vacuum, drying, rinsing, re-filtering, and re-drying;

d) separating the composite into components by a simple method.

2. A method of separating the carotenoids-LDH composite into carotenoid-enriched and LDH-enriched materials by the mechanical method of grinding and sieving is proposed. The method is based on the internal self-abrasion of the composite during grinding, while the harder LDH particles play the role of grinding bodies.

3. Retrieving carotenoids from fresh pumpkin pulp juice was carried out to obtain the carotenoids-LDH composite with different amounts of LDH. The complete and rapid sedimentation of the resulting composites and the ease of their separation from the mother liquor by vacuum filtration were revealed. X-ray diffraction analysis showed that the composite and products of its separation contain X-ray amorphous Zn-Al LDH, an oxide phase, and an amorphous phase of carotenoids.

4. The content (wt. %) and yield of carotenoids (mg/100 g of pumpkin pulp) in the separation products of the carotenoids-LDH composite samples obtained with different options of the LDH amount were determined by the dichloroethane extraction method. The efficiency of the composite separation process was proved. It is shown that for the optimal option of the Zn-Al LDH amount (calculated from the content of ascorbic acid), the content of carotenoids in the carotenoid-enriched material is 24.4 % and in the LDH-enriched material – 4.4 %. With the same optimal option of the LDH amount, it was revealed that the total carotenoid yield is 184.3 mg/100 g of pumpkin pulp, of which 155.4 mg/100 g in the carotenoid-enriched material and 28.9 mg/100 g in the LDH-enriched material. An increase and decrease in the LDH amount lead to a decrease in both the content and yield. It is suggested that these data need to be refined using a more selective method for determining carotenoids. A hypothesis was put forward about the chemical nature of the interaction between carotenoids and LDH in the composite due to the interaction of the system of conjugated π-bonds of carotenoids with Zn^{2+} d-orbitals in LDH.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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