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Catalytic Removal of Heavy Metals from Waste Water by Pumpkin Pectin-Containing Nanomaterials-Based Enzyme

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ABSTRACT

Over the past decades, the growing industrialization caused continuously increased release of heavy metal into environment. Through the food chain, heavy metals can be enriched in the body, causing serious harm. The removal and recycling of heavy metals are of great significance to environment protection, health concern as well as resource reuse. Pectic materials are available from a variety of natural sources and can be used as versatile adsorbents for heavy metal. Enzyme-based biocatalysts are one of the largest and commercially successful groups of catalysts. Integration of nanomaterials in the applications results in significant improvement of sensitivity, stability and other analytical characteristics. Thus, new functional nanomaterials are key components of numerous biosensors. However, due to the great variety of available nanomaterials, they should be carefully selected according to the desired effects. For the correction of pathological conditions of the human body, the prevention and rehabilitation of intoxication, the development of enterosorbents is necessary, one of which is various pectin products. Based on the conducted experiments, the regime parameters of obtaining pumpkin pectin-containing extract by the enzymatic method were established.

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INTRODUCTION

The problem of environmental pollution is one of the most important problems of mankind. Areas with a high anthropogenic load (large megacities), as well as areas of enterprises of heavy and light industry, are most prone to pollution. The chemical industries are important elements of the economy of the Republic of Kazakhstan, but they make a great contribution to the adverse environmental situation in the country [1-4]. One of the most pressing problems is environmental pollution with heavy metals (copper, iron, manganese, zinc, chromium, mercury, lead and cadmium). Lead and cadmium are highly toxic and have both acute and chronic effects on human health and the environment. The content of these

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metals in food, drinking water, and atmospheric air should be strictly controlled [5-8]. Lead belongs to the substances of the first hazard class and the World Health Organization is included in the list of priority pollutants [9-15]. Lead enters to the living organisms primarily from atmospheric air and soil. Lead has a wide toxic effect, including on the nervous, excretory, immune, cardiovascular and reproductive systems [16]. Environmental pollution with cadmium occurs due to the use of fossil fuels, work with metal-containing ores, as well as the burning of garbage [17,18]. Like lead, cadmium has acute and chronic toxicity, which leads to impaired function of the nervous system, kidneys, liver, and hematopoiesis. Lead and cadmium are cumulative poisons and accumulate in soil, water, plants and other living organisms. In vertebrate organisms, lead accumulates mainly in bones and teeth [19], and cadmium - in the liver and kidneys, but can be found in all organs [20]. It is known that birds are a sensitive indicator of the influence of environmentally unfavorable factors on the body of living objects, which can be indicated by the content of heavy metals in their body. For comparison, the content of heavy metals in feathers and internal organs of birds living in the city of Almaty (Kazakhstan) was determined. Depending on the pigeon habitat, the lead content in the feathers corresponded to 0.001–1.380 mg/kg, cadmium 0–0.058 mg/kg; lead content in internal organs (spleen, kidneys, liver) varies from 0.001 to 1.228 mg/kg; cadmium - from 0 to 0.031 mg/kg (table 1). In our studies, the total protein in the blood plasma of pigeons from ecologically safe zones was on average 50-55 g/l. With increasing environmental pollution and the accumulation of heavy metals in the body, there was a tendency to a decrease in total protein and the most pronounced hypoproteinomy in the range of 35-39 g/l was observed in pigeons captured from the Almaty-1 railway station [13,21].

There are several works have reported the application of nanomaterials based enzymes for various applications. The images shown in Fig. 1 and Fig. 2 present the application of nanomaterials based enzymes in applications such as sensors and catalysts. Among enzyme immobilization techniques, binding to a support and entrapment protocols need a carrier and, thus, there is a dilution effect on the enzyme activity during enzyme immobilization. However, the cross-linking method does not require any car-rier. Therefore, highly concentrated enzyme activity with high stability is obtained in the cross-linking of enzymes. Fig. 3 summarizes the common enzyme immobilization techniques used in enzyme-related applications.

In connection with a significant environmental pollution by a wide range of various pollutants, the issue of the search for biologically active and safe substances that correct physiological parameters and human homeostasis under conditions of increased environmental load is acute.

In the conditions of accumulation in the

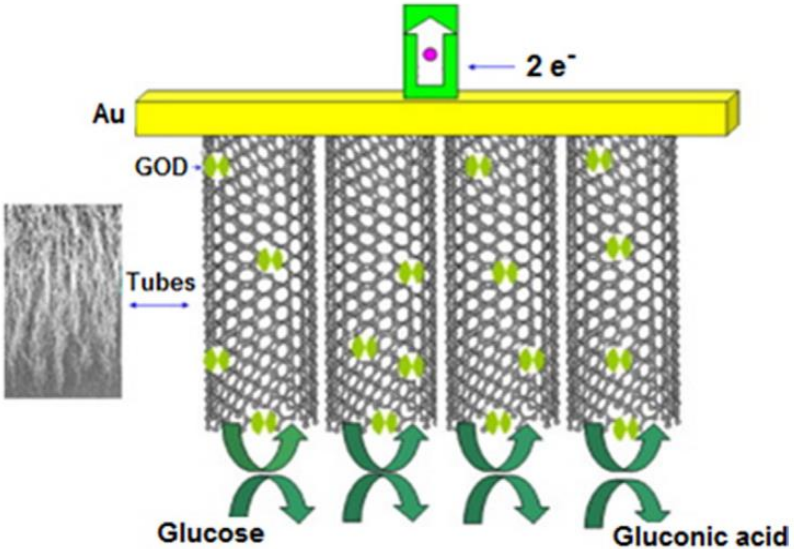

Fig. 1. Schematic illustration of the MWNT-based enzyme.

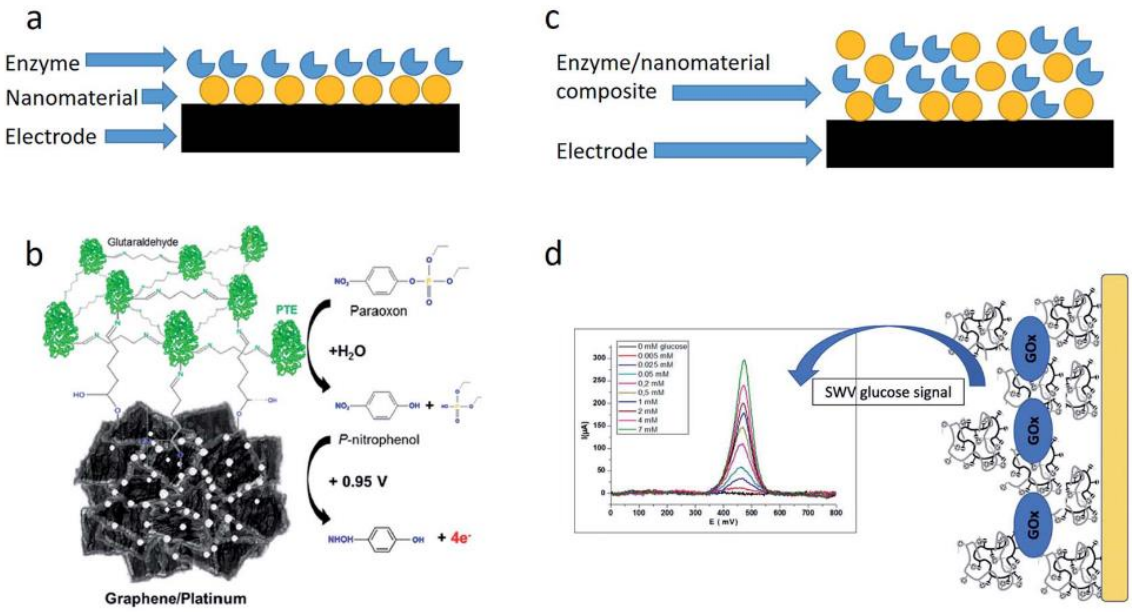

Fig. 2. Ways of embedding NMs in the enzyme-based biosensors. (a) Enzyme immobilization on the NM-modified electrode. (b) Schematic of the biosensor based on phosphotriesterase (PTE) immobilized via glutaraldehyde on the graphene surface with platinum nanoparticles. (c) Enzyme/NM co-immobilization on the electrode. (d) Schematic of the biosensor based on glucose oxidase encapsulated in a chitosan-kappa-carrageenan bionanocomposite.

environment of radioactive elements, heavy metal salts and pesticides that penetrate the human body, inexpensive melon products with a high content of pectin, carotene, and dietary fiber are of particular interest.

The World Health Organization (WHO) recognized pectin as an absolutely toxicologically safe product and is recommended as a corrector of human health. Recent studies have shown that it is more efficient to use substances contained in natural food products: they do not cause side effects and give a protective effect. Such substances include pectin, which has a beneficial effect not only under the conditions of acute exposure to metals, but also with their long-term intake into the body, which is typical for the environmental load of residents of industrial regions and the modern megapolis [21]. The main effect of the

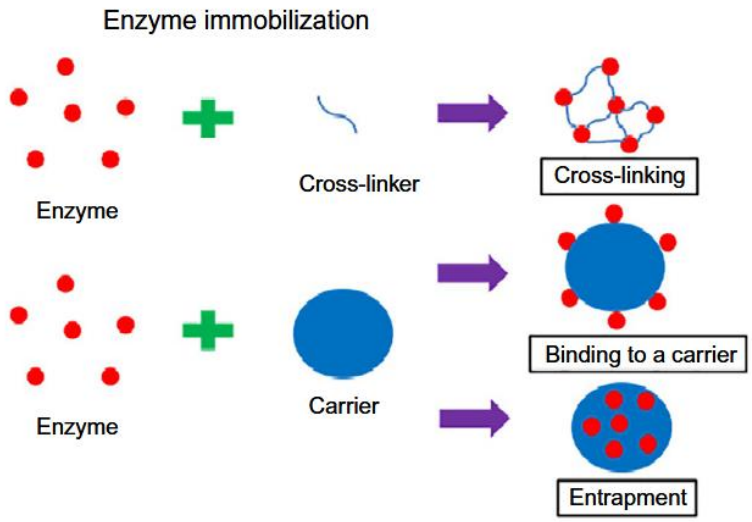

Fig. 3. Enzyme immobilization techniques.

Table 1. The content of heavy metals in the organs of blue pigeons from different places in Almaty, Kazakhstan (mg / kg)

Researched zones	Pb feathers	Cd feathers	Pb spleen	Cd spleen	Pb kidneys	Cd kidneys	Pb liver	Cd liver
Kok-Tobe	0.001± 0.001	0	0.003± 0.000	0.001± 0.000	0.001± 0.000	0	0.001± 0.000	0.001± 0.000
Tastak microdistrict	0.122 * ± 0.001	0.019 ±0.004	0.089 ±0.001	0.019 ±0.001	0.076±* 0.047	0.017±* 0.001	0.083±* 0.001	0.007±* 0.001
Almaty - 1	1.380 * ±0.101	0,058 * ±0,002	1.146 * ±0.094	0.031 * ±0.002	1.194 * ±0.073	0.028 * ±0.002	1.228 * ±0.110	0.010 * ±0.002

* significantly at p <0.05

therapeutic effect of pectin is associated with the peculiarities of its chemical structure - the presence of chemically active free carboxyl groups and alcohol hydroxyls, which contribute to the formation of strong insoluble complexes with polyvalent metals, the so-called chelates, which remove heavy metals and radionuclides from the human body [22].

Pectin consists of galacturonic acid residues and galacturonic acid methyl esters forming linear polysaccharide chains and is usually classified by degree of esterification (DE). HE (highly esterified) pectin has an DE above 50, forms gels and structured liquids under conditions of high acidity and low activity of water (for example, a high concentration of sugar, sorbitol, or glycerol). LE (low esterified) pectin has an DE below 50, which forms jelly in the presence of a certain amount of calcium or other divalent cations. Pectins are widely used in the food and pharmaceutical industries, as dietary fibers, have properties that are beneficial to the human body. Daily it is recommended to eat 10-14 grammectin. Pectin improves the functioning of the stomach and intestines - it stabilizes the growth of beneficial microflora and fights against fermentation and rotting, lowers cholesterol - pectin binds about 15% of excess cholesterol in one meal, preventing it from entering the bloodstream. Helps improve skin condition with various dermatological diseases - pectin substances remove allergens and toxins that cause skin problems, allergies and rashes.

The use of pectins is the prevention of cancer, helps to reduce weight, reduce the harmful effects when taking a large number of drugs and is recommended when living in an area with high radiation [23-28]. The aim of this study was to establish the regime parameters for the extraction of pumpkin pectin-containing extract by the enzymatic method.

MATERIALS AND METHODS

Based on preliminary monitoring studies of materials for deep processing of melons, we selected a zoned variety of pumpkin “Karina” of domestic production. With the help of a laboratory automatic squeezer, such as “Scarlettjuice extractorsc-015”, the liquid phase was separated from the pumpkin variety “Karina” and obtained pomace. The study of technological modes for obtaining pectin-containing extract from dry pumpkin pomace (temperature, doses of the enzyme preparation, pH) was carried out by using the enzyme preparation Pectinase from Aspergillus niger - Sigma-Aldrich and working out its optimal doses at the highest concentration of pectin in the extract. At the same time, before the introduction of the enzyme preparation, dry pumpkin pomace is pre-swelled in water at a temperature of 20-60 ˚C for 12-15 hours. The enzymatic treatment of plant raw material is carried out at temperature in the range of 33.0 to 41.0 ˚C and the medium pH in the range of 5.5 to 8.0 with a weight ratio of raw materials and water in the range of 1: 10 to 1:13, regulating the natural medium with 0.1

M NaOH solution and 1.0 M acetic acid solutions and certain amount of nanomaterial substrate. In this case, the most optimal pH environment and incubation temperature will be considered the one at which the largest amount of pectin is released from the pumpkin pomace in the shortest time. The duration of enzymatic processing of plant raw materials will be determined by conducting enzymatic extraction for 1 to 6 hours. In this case, the most optimal pH medium and incubation temperature will be considered the one at which the greatest amount of pectin is released from pumpkin pomace in the shortest time. The duration of the enzymatic treatment of plant materials will be determined by conducting enzymatic extraction for 1 to 6 hours.

Study of optimal doses of enzyme preparations for obtaining pectin-containing extract

The optimal working dose in the technology of obtaining pectin from pomace will be considered the spent dose of the enzyme preparation, which in a relatively short time will contribute to the maximum release of pectin from the product. For better preservation of the pectin extract, it is concentrated by vacuum evaporation to the content of soluble solids of 20-25% and the pectin content of 2.0-3.0%. The experiments were performed in three-fold repetition.

The degree of esterification is the ratio of the number of esterified carboxyl groups to the total content of carboxyl groups in pectin (esterified and non-esterified). The lower the degree of pectin esterification (more free carboxyl groups), the higher its detoxifying activity [11]. To determine the analytical characteristics of the pectin used method of conductometric titration (conductivity meter HI 8733 HANNA company), jelly-forming ability of pectin was determined using the instrument Sosnowski. The degree of esterification was determined using the titrimetric method [29]. The content of free carboxyl groups was determined by titration of a solution of a pectin-containing preparation, and after saponification - esterified carboxyl groups.

The degree of pectin esterification (DE) is calculated using the formula:

$$\text{Сз} = \frac{K_m}{K_0} 100 \quad (1)$$

where: K_m-mole fraction of methoxyl groups;

K_0 is the mole fraction of free and esterified carboxyl groups.

Titrimetric determination of the content of carboxyl groups in pectin.

Determination of the content of free carboxyl groups according to OST 18-62-72.

The content of free carboxyl groups of K_c,%, is calculated by the formula:

$$K_c = \frac{V_{NaOH}}{m_{pectin}} \cdot 0{,}0045 \cdot \frac{V_{colb}}{V_{aaliquotes}} \cdot 100\% \quad (2)$$

Determination of the mass fraction of free and methoxylated carboxyl groups

The content of free carboxyl groups (Cs,%) is calculated using the formula:

$$K_c = \frac{a}{G_1} 0.45 \quad (3)$$

where: a is the amount of 0.1 N NaOH solution spent on titration, ml;

1 mL of 0.1 M NaOH solution corresponds to 0.0045 g COOH.

In the same solution, the number of methoxylated carboxyl groups is determined. After determining the content of free carboxyl groups, a 10 mL 0.5 M NaOH solution is added to the neutralized sample from a burette. The flask is closed and left for 2 hours at room temperature for hydrolysis of methoxylated carboxyl groups. Then 10 mL of 0.5 M HC1 is added to the solution from the burette and the excess of the latter is titrated with 0.1 M NaOH.

The amount of 0.1 M NaOH spent on the second titration corresponds to the number of esterified groups (CE, %) in the test sample, which is calculated by the formula:

$$K_e = \frac{b}{G_{nb}} 0.45 \quad (4)$$

where: b is the amount of 0.1 M NaOH spent on the second titration;

G_{nb}-suspension of washed and dried pectin powder, g.

To calculate the number of methoxylated carboxyl groups, an adjustment must be made for acetyl groups, which are also hydrolyzed under these conditions.

The number of methoxyl groups (Km, %)

adjusted for acetyl groups is:

$$K_M = K_e - A_c \quad (5)$$

where: A_c – the number of acetyl groups %.

Determination of the mass fraction of acetyl groups in pectin. A suspension of pectin (1.0 g) is placed in a measuring flask with a capacity of 50 ml, add 25 ml of 0.6% NaOH solution and leave for 6...8 h. Then the contents of the flask are adjusted to the mark with distilled water. Take 20 ml of the solution into the distillation flask, add 20 ml of the $MgS0_4$ solution. Select 1 ml of distillate and titrate in the presence of phenolphthalein 0.1 N NaOH solution. In parallel, an idle experiment is performed with 20 ml of solution and 20 ml of distilled water. The difference between titrations corresponds to the number of acetyl groups (A_c, %), the content of which is calculated using the formula:

$$\mathrm{A}_c = \frac{43.04\mathrm{C}}{G_1 100} \quad (6)$$

where: C - the difference between the amount of 0.1 M NaOH solution spent on titration in the experiment with a hitch and in the idle experiment, mL; 43.04-equivalent mass of acetyl groups.

The sugar content was determined according to State Standard Specification 8756.13-87 [30]. The method is based on the ability of carbonyl groups of sugars to reduce copper oxide(II) to copper oxide (I) in an alkaline environment. When dissolved by iron-ammonium alum, the formed copper oxide (I), oxidizing to copper oxide (II), reduces iron (III) to iron (II), the amount of which is determined by titration with a solution of potassium permanganate. Carotene content was determined according to State Standard Specification R 54058-2010 [31]. The method for determining the mass concentration or mass fraction of carotenoids, including total carotenoids and their individual fractions (carotenes, cryptoxanthine and xanthophyll esters) is based on the extraction of carotenoids from a sample or precipitate previously obtained by processing the sample with Carreza I and Carreza II solutions, followed by purification of the isolated preparation with petroleum ether and spectrophotometric determination of mass concentration or mass fraction of carotenoids. The fractions of individual carotenoids (from the total carotenoid content) are determined by spectrophotometric measurement in the fractions obtained during chromatographic separation of the extract. Determination of water-soluble vitamins in raw materials and products was performed by capillary zone electrophoresis on the capillary electrophoresis system "Kapel 105 M" (Russian Federation, Lumex). Standard solutions of vitamins B12, B6, PP, and C were used as a control sample [32]. The content of Na, K, Mg, Ca, Fe, Cu, and Zn was determined by atomic absorption spectroscopy (AAS) using a QUANTUM-Z. ETA-T electric atomization spectrometer (RF, Cortex) with software. The measurement method is based on the measurement of the absorption (optical density) of the atomic vapor of the element being determined, obtained by electrothermal atomization of the sample in the graphite furnace of the spectrometer. Measurements of the optical density of atomic vapor are made on the resonant spectral line of the element emitted by the corresponding lamp with a hollow cathode. Preparation and conduct of atomic absorption measurements of heavy metals was performed in accordance with the ND [33].

Vitamin E (tocopherol) and vitamin A (retinol) were determined directly from the extracted oil by high-performance liquid chromatography (HPLC) on an Agilent 1200 high-performance liquid chromatograph (Agilent Technologies, USA) equipped with a Zorbax 300SB-C18 column: size 4, 6x250 mm 5 µm (Agilent,Zorbax) and an Agilent 1200 G1321A FLD fluorescent detector. The tocopherol content was calculated based on the areas of tocopherol peaks in the sample, depending on the tocopherol peak area of the standard tocopherol solution [34,35]. The operating conditions for determining vitamin A were as follows: mobile phase-a mixture of acetonitrile, methyl alcohol, and methylene chloride in a volume ratio of 50:45:5., flow rate 0.7 ml / min, excitation 295 nm, radiation 330 nm., 20 µl 1% solution of raw extracted oil. Quantitative analysis was performed using an external standard method using the area of retinol peaks [36].

The carbohydrate composition was determined by high-performance liquid chromatography. Equipment: high-performance liquid chromatograph "Agilent 1200" (Agilent Technologies, USA), temperature-controlled chromatographic column Aminex YPX-87P size 300x7.8 mm 5 µm (Bio Rad) and refractometric detector Agilent 1214 Infinity G1362A RID. The

Table 2. Results of determining the quantitative yield of the liquid phase-juice, pomace and seeds of pumpkin Karina variety at the stage of technical ripeness

Kind of pumpkin	amount	Juice output		Seed output		Pomace output		Losses	Dry pomace (pulp)	
	g	ml	%	g	%	g	%	%	g	%
Karina	2741	561	20.47	244	8.9	1845	67.31	3.32	335	12.22

analysis conditions were as follows: mobile deionized water (by volume), flow rate 0.7 ml/min. the carbohydrate Content was calculated based on the peak areas of calibration solutions of a mixture of sugars (sucrose, glucose, fructose) [37].

Determination of the complex forming ability of pectin was carried out by the method of conductometric titration

The essence of this method is that a certain amount of lead ions is introduced into the medium containing pectin. After binding lead with pectin, the amount of non-pectin-bound lead is determined by reverse titration. The control experiment is carried out similarly, but instead of pectin or pectin extract, 20 ml of distilled water is added to the flask.

As a result of the experiments conducted to determine the complexing ability of pectin obtained from pumpkin pomace, the mass of lead was determined in the analyzed and control experiments, and the resulting results were used to calculate the complexing ability of the pectin-containing product in milligrams of lead ions per gram of pectin.

RESULTS AND DISCUSSION

It is known that Kazakhstan has a sufficient raw material base and a large assortment of melons, among which the high content of pectin is distinguished by the variety of pumpkin Karina. A quantitative and qualitative analysis of the components of pumpkin in laboratory conditions was carried out. The obtained research results are presented in Table 2 and Fig. 3. For the purpose of preservation, the resulting raw pumpkin pomace was dried in a drying Cabinet at a temperature of 45-50 0C to a humidity of 8%.

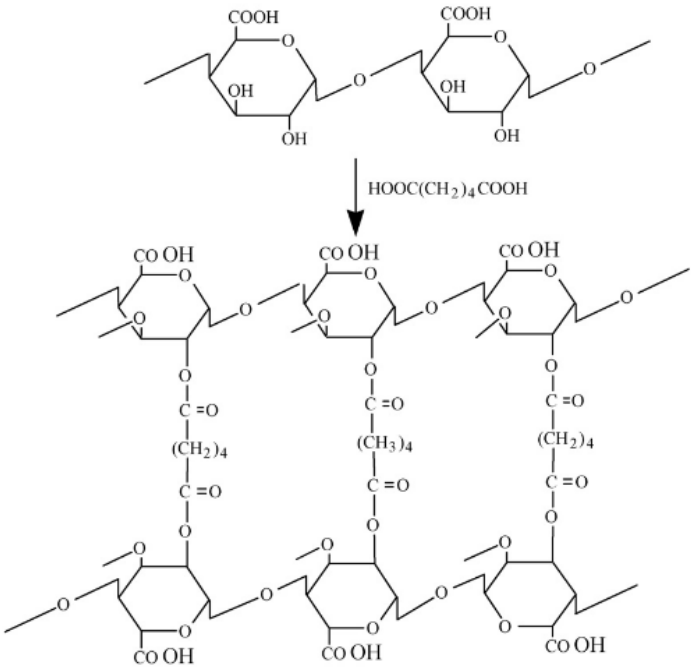

Fig. 4. Cross-linking reaction between saponified pectin and adipic acid.

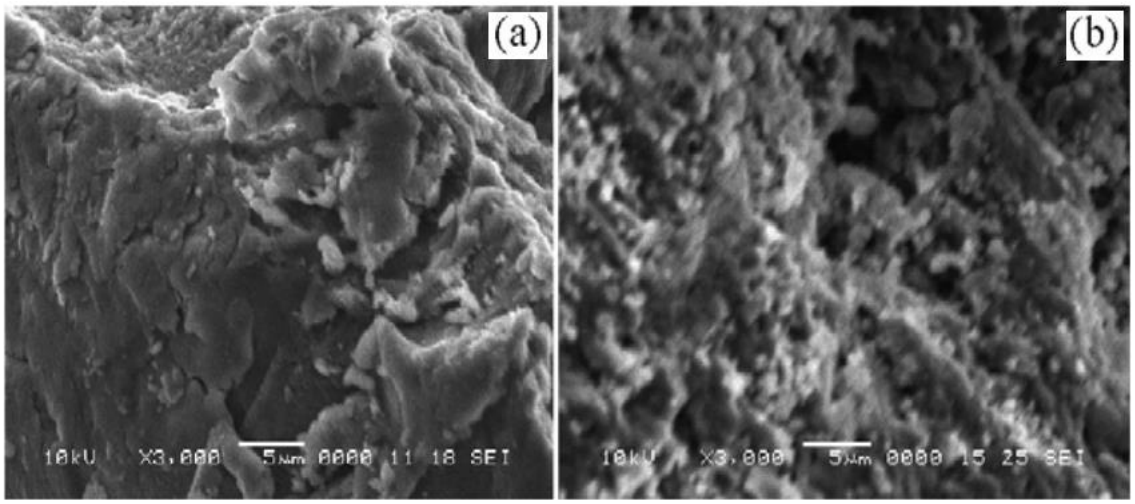

Fig. 5. EM images (10 kV) of extracted pectin (a) and modified pectin (b).

According to Table 2, the yield of the liquid phase in the pumpkin Karina variety was at a total weight of 2741.0 g only 561±2.0 ml, which is 20.47±0.03%, raw pomace -1845±2.0 g, respectively, 67.31±0.01%, seed output-244±2.0 g or 8.9±0.01%. The total loss was 3.32±0.01%. After drying, the weight of dry pomace was 335g, which is -12.22% of the original weight.

Cross-linking reaction can reduce pectin's hydrability (i.e. distensibility) and enhance stability of this absorbent. In this study, adipic acid was chosen as cross linkers instead of common cross linkers (e.g. epichlorohydrin), because epichlorohydrin has a good cross-linking performance, but it has great toxicity and easily leads to side reactions. The cross-linking reaction between saponified pectin and adipic acid is shown in Fig. 4. The carboxy groups, displayed with capital are potential functional adsorption units..

The scanning electron micrographs of pectic substances are displayed in Fig. 5. The modified pectin (Fig. 5b) is of more porose than that of the extracted pectin shown in Fig.5a and may offer an easy access for ion diffusion. The post-extraction process obviously improved loading capacity of pectin.

To isolate pectin, the enzyme preparation Pectinase from Aspergillus niger, which is well used on apple raw materials, was used. When

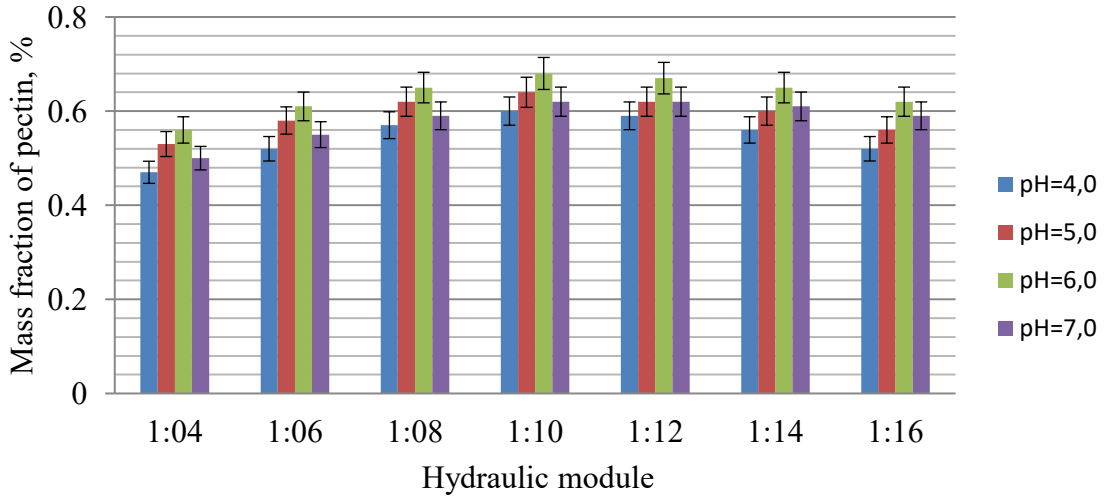

Fig. 6. Influence of pH-medium and hydraulic module on the degree of output of pectin.

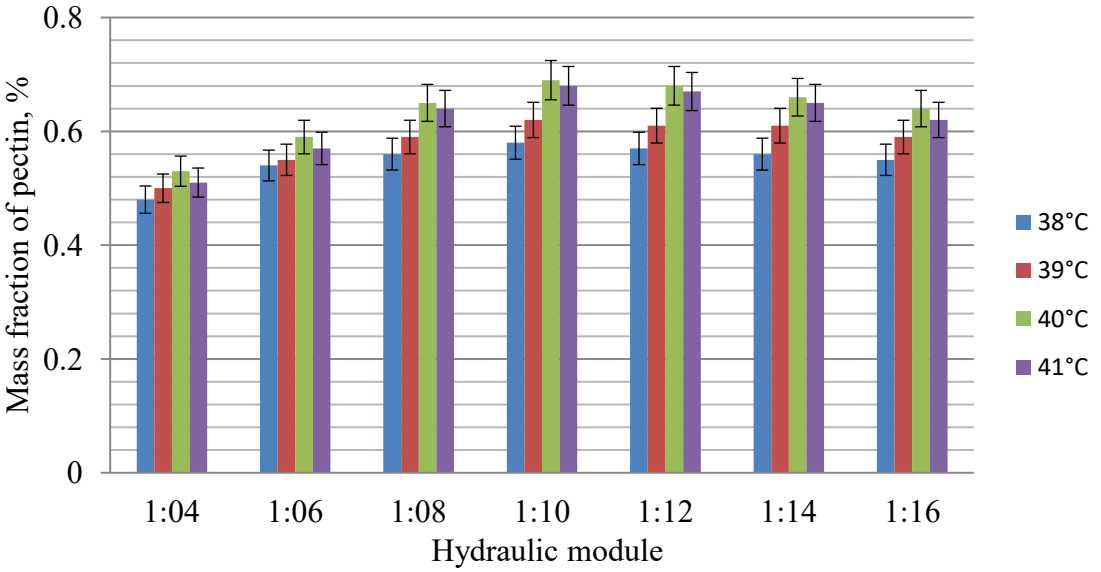

Fig. 7. Influence of temperature and hydromodule on the degree of pectin yield.

studying the technological modes (hydromodule, temperature, pH of the medium) for obtaining pectin-containing extract from pomace of pumpkin of the Karina variety, before the introduction of the enzyme preparation, the raw material was pre-swelled in water at a temperature of 48-50 ˚C for 12-15 hours. Pectin extracted from pre-swollen raw materials has higher quality indicators, in addition, to obtain a pectin-containing extract from pumpkin pomace, the method does not use aggressive agents and is environmentally friendly. To work out the technological modes for obtaining pectin-containing extract from pomace of pumpkin of the Karina variety by an enzyme method, the optimal temperature regime, pH-media and hydromodule for obtaining pectin-containing extract were worked out.

Enzymatic treatment of plant raw materials was carried out with a minimum dose of 0.5% at temperature ranges from 38.0 to 41.0 ˚C, pH of the medium from 4.0 to 7.0, with the weight ratio of raw materials and water in hydromodules: 1:4, 1:6, 1:8, 1:10, 1:12, 1:14 and 1:16, regulating the original natural environment of the extract pH=5,3-5,9 and 0.1 M NaOH and 1H solutions of acetic acid. Enzymatic treatment was performed for 3 hours.

At the same time, the most optimal pH-medium, temperature and hydromodule of incubation will be considered the one at which the largest amount of pectin will be released from pumpkin pomace for a certain equal exposure time.

At the end of fermentation, the extract was filtered and centrifuged at 8000 rpm for 15 minutes to purify and clarify the extract, after which the enzyme was inactivated in the extract at a temperature of 75-77 ˚C for 30 minutes, followed by cooling of the extract. The results of studying the optimal technological modes (hydromodule and pH-medium) for obtaining pectin-containing extract from pomace of pumpkin of the Karina variety are shown in Fig. 6.

A study of optimal technological modes of a hydromodule and pH medium, upon obtaining by the enzymatic method (enzyme Pectinase from Aspergillus niger) pectin-containing extract from pomace of pumpkin of the Karina variety, it is established that the optimal medium pH in the enzymatic extraction of pectin from pomace pumpkin Karina is the medium pH at 6.0 and the hydraulic module of 1:10 (the content of pectin 0.68 %), as medium pH: 4,0, 5,0, 6.0 and 7.0 and the water ratios 1:4, 1:6, 1:8, 1:12, 1:14 and 1:16 of the resulting extracts contained pectin at lower concentrations.

The results of studying the optimal technological mode (temperature) for obtaining pectin-containing extract from pomace of pumpkin of the Karina variety are shown in Fig. 7.

As a result of studying the optimal technological mode: extract temperature, it was found that the optimal extract temperature for enzymatic

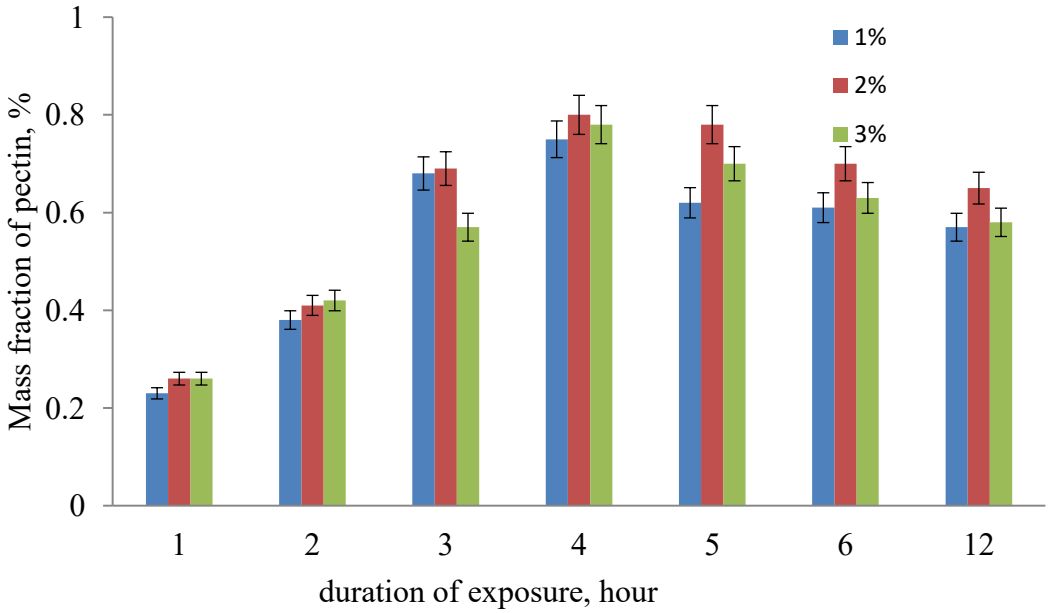

Fig. 8. Effect of exposure duration and concentration of the enzyme preparation on pectin yield.

extraction of pectin from pomace of pumpkin of the Karina variety is a temperature of 40-41 ˚C (content of 0.68-0.69%), which is unfavorable for many microorganisms, which undoubtedly has a positive effect in the technological process, with a hydromodule of 1: 10, while at temperatures

Table 3. Mass fraction of vitamins and minerals in terms of absolutely dry weight

№	Name of indicator	Pumpkin pomace	Pumpkin pectin extract
1	Carotene content, mg / kg	4,50	3,13
2	The content of vitamin A, mcg	77,00	53,9
3	The content of vitamin B_{12}, mg	0,17	0,12
4	The content of vitamin B_6, mg	1,23	0,84
5	Содержание витамина С, mg	25,00	11,00
6	The content of vitamin E, mg	1,23	0,86
7	The content of vitamin PP, mg	2,15	1,50
8	Na content, mg	12,00	8,40
9	K content, mg	629,00	440,10
10	Mg content, mg	43,00	30,10
11	Ca content, mg	77,00	54,00
12	P content, mg	75,00	50,40
13	Fe content, mg	1,23	0,85
14	Cu content, mcg	555,00	375,50
15	Zn content, mg	0,74	0,51

Table 4. Mass fraction of sugars, % in terms of absolutely dry mass.

№	Products	total sugar W%	fructose W%	glucose W%	sucrose W%
1	Pumpkin pomace	26,3	5,9	3,1	15,3
2	Pumpkin pectin extract	18,0	4,1	2,2	10,7

of 38-39 ˚C and in hydromodules 1:4, 1:6, 1:8, 1:12, 1:14 and 1:16 obtained extracts contained pectin in a lower concentration (Fig. 7). The results of determining the optimal dose and exposure time for the enzyme complex during enzymatic extraction of pectin from pomace of pumpkin of the Karina variety are shown in Fig. 8.

When determining the optimal dose and exposure time for the enzyme complex during enzymatic extraction of pectin from pomace of pumpkin of the Karina variety, it was found that at a recommended temperature of 40-41 ˚C, pH=6.0, a water module of 1:10, the optimal dose of the enzyme during enzymatic extraction of pectin from pomace of pumpkin of the Karina variety, the dose is 2.00% and the exposure time is 4 hours (pectin content is 0.78 - 0.80 %).

As a result of establishing rational modes for the extraction of pectin substances from pomace of pumpkin of the Karina variety (temperature 40-41 ˚C, the dose of the enzyme preparation Pectinase from Aspergillus niger was 2.0%, the pH of the medium was 6.0, and the exposure time was 4 hours), a pectin-containing extract was obtained. The extract obtained was concentrated by vacuum evaporation using an apparatus of the brand RV 05 basic 2-B manufactured by Belakvilon, Republic of Belarus, at a temperature of 58-60 ˚C and a vacuum discharge of 0.5-0.7 atm., to a pectin content of 2.40-2.52 ± 0.02 % and soluble solids 22.0-25.0 ± 0.02%.

The results of biochemical parameters of raw materials and pectin extract are shown in Table 3. We found that pumpkin pomace contains a large amount of carotene, vitamins A and C, as well as minerals: K, Cu, Mg, Ca, P, about 75-80% of which during processing, they transfer to pectin extract.

Since one of the quality criteria for pectin is the content of pure pectin in the commercial sample, additional studies of raw materials and pectin products for the presence of sugars, which are ballast substances in relation to pectin, were conducted. Experimental data on the total content and fractional composition of sugars are shown in Table 4.

The data table shows that the samples of the studied raw materials and pectin extract contain sugars. This makes it necessary to prepare these raw materials for hydrolysis-extraction of pectin substances. To assess the quality indicators of pectin extract from pumpkin pomace of the "Karina" variety, its analytical characteristics of functional orientation were determined. The results of analytical characteristics of pectin extract from pumpkin pomace, its gel-forming and

Table 5. Quality of pectin extract from pumpkin pomace % in terms of absolutely dry weight.

№	Name of indicator	Quality indicators of pectin-containing extract
1	The content of free carboxyl groups,%	3,0
2	The content of methoxylated carboxyl groups,%	1,9
3	The degree of esterification,%	34,7
4	The content of methoxy component,%	4.8
5	Acetyl content,%	0,94
6	The content of the polyuronide component,%	33,7
7	Complexing ability, mg Pb^{2+} / g	290
8	Jelly Strength, ^{0} SAG	95

complexing ability are shown in Table 5.

A study of the analytical characteristics of pumpkin pectin showed that the main properties of pectin substances, which determine a wide area of their application in the food industry, is gel-forming ability: the content of methoxyl groups in the variety Karina is 4.8%, the uronide component is 33.7% (Karina), and the content of acetyl groups (less than 1.0, i.e. 0.94).

Previous studies of Russian scientists showed that the complexing ability of pectin from the peel of lupine seeds obtained by extraction with citric acid solution and the percentage of lead ion binding were 141.3 mg / g and 20.3%, respectively. Complexation proceeds according to kinetics of the first order. The possibility of using the obtained pectin as a detoxicant has been shown [37]. Thus, the results of our studies (Table 5) can be concluded that the content of free carboxyl groups in pumpkin pectin extract corresponded to 3.0%, while the degree of esterification was low and amounted to 34.7%, which corresponds to the high value of the complexing ability of the pectin-containing extract from squeezed squash - 290 mg Pb^{2+}/g. The high complexing ability of pectin confirms the possibility of its use for nutrition of people living or working in an environment contaminated with heavy metals, radionuclides [24, 27].

CONCLUSION

The present work studied the catalytic property of the as-prepared extract. Analysis of literature indicated that among the many problems currently facing humanity, one of the first places is the problem of environmental pollution by various chemicals - products of technogenesis, among which heavy metals occupy a significant place. In the present work, rational modes for the extraction of pectin from pomace of pumpkin of the Karina variety were established (temperature 40-41 ˚C, the dose of the enzyme preparation Pectinase from Aspergillus niger was 2.0%, the pH was 6.0, and the exposure time was 4 hours). The obtained extract was concentrated by vacuum evaporation using an apparatus of the brand RV 05 basic 2-B at 58-60 ˚C and a vacuum discharge of 0.5-0.7 atm. The pectin content was 2.40-2.52 ± 0.02% and soluble solids were 22.0-25.0 ± 0.02%. The pectin substances obtained by enzymatic method possessed high quality indicators. The degree of esterification 34.7%, complexing ability 290 mg Pb^{2+}/g were recommended for use in the food industry in functional foods with detoxifying properties for the prevention and reduction of toxicants in the human body.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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