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# Beet pulp dietary fiber exposed to an extremely low-frequency electromagnetic field: detoxification properties

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**Abstract:**

*Introduction.* The lack of dietary fiber in the Russian people diet contributes to the development of various diseases. In this regard, it seems worthwhile to enrich foods with dietary fiber obtained from various types of raw materials. In our experiments, we used beet pulp. This study aimed to develop a technology for obtaining combined dietary fiber using the electrophysical method and evaluate its detoxification properties.

*Study objects and methods*. Study objects were pectin substances and combined detoxicants from beet pulp obtained by extracting with succinic acid with and without an extremely low-frequency electromagnetic field (ELF EM) treatment. The profiles of combined detoxicants and pectin substances were identified by IR-Fourier spectrometry. Beet pectin, beet cellulose, and their combined detoxicants were tested for complexing (binding) capacity with respect to lead ions ($Pb^{2+}$). For this, we applied the trilonometric method with some modifications.

*Results and discussion*. The analysis of the absorption bands of carboxyl groups carbonyls revealed the presence of free carboxyl groups in the combined detoxicants. The combined detoxicant with a 1:0.5 ratio of cellulose and pectin substances showed a high complexing (binding) capacity (601 mg/$Pb^{2+}$) with respect to lead ions ($Pb^{2+}$).

*Conclusion*. We developed a technology for producing combined detoxicants with a high complexing capacity with respect to lead ions analysed microstructures of gels obtained during the interaction between the combined detoxicant and lead acetate solution.

**Keywords:** Pectin, cellulose, beet, detoxicant, extraction, IR spectra, binding capacity, complexation

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## INTRODUCTION

In 2012, the General Assembly of the World Health Organization (WHO) adopted the Global action plan for the prevention and control of noncommunicable diseases 2013–2020, supported by the health ministers of all the member states. It promoted the idea that people's health is determined not only by working conditions, but also by social and anthropogenic factors.

Today, Russian people consume too many simple carbohydrates and animal fats and not enough fruits and vegetables. The lack of dietary fiber contributes to various diseases, including diabetes, colon cancer, hernia of the oesophageal opening of the diaphragm, obesity, and gallstone disease. A sufficient amount of dietary fiber helps prevent dental caries and reduces the level of cholesterol in the body. Dietary fiber is involved in metabolic processes, controls gastric emptying, binds heavy metals and carcinogens and excretes them from the body [1].

Therefore, using dietary fibers obtained from various types of raw materials appears to be a promising way of enriching foods. Several recent studies have aimed to create foods with plant-based dietary fiber, including fiber from non-traditional raw materials [2–4].

Dietary fiber can be produced from beet pulp – a by-product from the processing of sugar beet at sugar

factories and table beet at canning factories [3]. Beetroot is a good source of dietary fiber that is used as a dietary supplement in various food products [5, 6].

Beet pulp is the cheapest pectin-containing raw material [7]. There are over 130 pulp drying stations at sugar factories in the CIS (Commonwealth of Independent States) countries. Their total capacity is 10 000 t of dried pulp per day. Thus, with an average yearly operation for 100 days, they can produce at least 800 000 t of dried pulp per year [8].

The cell walls of sugar beet pulp contain such dietary fibers as cellulose, hemicellulose, and pectin substances [9]. These compounds bind together, forming a so-called "pectin and cellulose complex" in a plant cell. However, there are no conditions for their detoxification properties to fully manifest, since their functional groups – involved in complexation (binding) reactions and ion-exchange processes – are difficult to access [10, 11].

Cellulose is an insoluble dietary fiber with almost no functional groups allowing for the sorption of heavy metal ions during complexation. Due to its mechanical detoxification properties, cellulose sorbs mainly high molecular weight compounds.

Pectin substances are soluble dietary fibers. Their detoxification properties are determined by free functional carboxyl groups [12]. Due to these groups, pectin substances sorb low molecular weight compounds and participate in complexation with respect to heavy metal ions [13, 14].

It seems worthwhile to recover pectin substances separately from cellulose, when destroying the pectin and cellulose complex, to release the functional groups of their molecules. Since they manifest different binding activity with respect to heavy metal ions, as well as different detoxification properties, it is advisable to obtain a combined detoxicant from these substances.

A number of studies, both in Russia and abroad, have aimed to optimise the production of dietary fiber with detoxification properties from beet processing by-products.

One of the studies proposed a technology of treating sugar beet pulp with high-voltage electric discharges (HVED) prior to pectin extraction [15]. After the HVED pre-treatment, pectin was extracted in acidified water with varied pH and temperature. The results showed an increase in pectin yield from 42.6% for untreated sugar beet pulp to 53.4% for HVED-treated sugar beet pulp under optimal extraction conditions (t = 90°C, pH = 2, duration = 1 h). The Fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry showed similar functional groups and chemical composition between the standard of sugar beet pectin and the extracted molecules from untreated and HVED pre-treated sugar beet pulp.

Another study investigated the effect of beet pulp particle size on the extraction and physicochemical properties of pectin [16]. Reducing particle size from 406.33 to 24.93 μm increased pectin yield from 15.81 to 20.50% and the content of galacturonic acid from 38.51 to 59.97%. Larger particle size enhanced pectin's rheological properties (kinematic and dynamic viscosity, and activation energy). All pectin solutions showed the same linear viscoelastic properties. Beet pulp particle size affected the dynamic moduli of extracted pectin.

A method of gradual ethanol precipitation of sugar beet pectin from an acidic extract was offered by [17, 18]. The procedure was developed to purify sugar beet pectin from a pectin-containing aqueous extract. Five fractions with different chemical and molecular characteristics were obtained by gradually increasing the concentration of ethanol in the precipitating medium from 50 to 80%. The analysis of chemical and macromolecular characteristics of the fractions indirectly showed that the ability of pectin to dissolve in the ethanol-water binary mixture strongly depended on the polymer structure. The fractions rich in neutral sugars were precipitated at relatively high concentrations of ethanol, probably due to increased interaction between pectin chains and solvent molecules. The authors found that gradual ethanol precipitation was more selective in relation to pectin's structural features and surface properties than single-stage ethanol precipitation.

Guo *et al.* established the efficacy of purifying sugar beet pectin from non-pectin components by metal precipitation [19]. To assess the selectivity of copper ions in relation to binding pectin or non-pectin compounds, two fractions were fractionated from sugar beet pectin, namely copper-precipitated pectin and copper-unprecipitated pectin. The chemical analysis revealed certain structural differences between the fractions. In particular, copper-unprecipitated pectin contained a markedly higher amount of neutral sugars and proteins, but a lower content of galacturonic acid and acetyl ester groups, compared to copper-precipitated pectin. However, there was no appreciable difference in the degree of methylation. In addition, AFM analysis showed that copper-precipitated pectin displayed branched fibrous structures, while copper-unprecipitated pectin had distinguishable granular shapes. The authors concluded that copper ions selectively bound the anionic regions among pectin chains, thereby separating pectin saccharides from non-uronide compounds, i.e. low molecular weight carbohydrates and free proteins.

The process of ethanol precipitation of sugar beet pectin with and without counter ions was investigated by [20]. In aqueous solution, when cations bound to sugar beet pectin, the negative charges on the pectin chains reduced and the hydrodynamic radius of the whole polymer decreased. Cation-bound pectin were more prone to ethanol precipitation because they were less solvated by water molecules. In the absence of cations, partially-ionized beet pectin actively interacted

with water molecules at the initial precipitation pH (I-pH) range of 3.26–2.83, which made it difficult to precipitate pectin by adding ethanol. However, in the presence of cations, the precipitation of sugar beet pectin was straightforward due to cation binding and ethanol dehydration. Protonated pectin and their salts were obtained. The authors found that no conformational transition occurred in cation-bound pectin after ethanol precipitation. This indicated the importance of the electrostatic nature of the caution-beet pulp interaction during the precipitation process.

The effect of pH on the ethanolic precipitation of sugar beet pectin was studied by [21]. Sugar beet pectin was precipitated from a purified extract in a 75% ethanol solution at an initial extract pH (I-pH) ranging from 2.0 to 4.5. The authors studied the effect of I-pH on pectin yield and pectin-cation interactions. The lowest pectin yield was obtained by precipitating the acidic pectin extract with ethanol at I-pH 4.5. Pectin yield increased to a maximum value as I-pH decreased from 4.5 to 3.0. The results indicated that decreased electrostatic repulsion between pectin chain segments enhanced pectin chain-chain interactions, thereby improving the precipitation effect. A decrease in pectin yield was observed as I-pH decreased below 3.0. The cation content of various samples was measured by high-performance cation-exchange chromatography in order to determine the content of pectin-bound cations during precipitation. The cation content and degree of cation binding were measured for pectin precipitated at I-pH 3.0, 2.5, and 2.0. The results revealed that a decrease in pectin yield accompanied a decrease in cation-pectin interactions. This suggested that the precipitation of beet pectin substances from an aqueous extract involved complex interactions between cations, solvent molecules, and pectin chain segments. The larger precipitation effect observed with divalent ions, compared to monovalent ions, might be due to enhanced inter-chain interactions between pectin molecules, probably via the formation of intermolecular bonds.

Most developments in this field offer ways to increase dietary fiber yield from raw materials. Fewer studies aim to develop technologies for improving its complexing capacity, which is becoming increasingly relevant today.

Important risk factors for many diseases include disturbed nutrition, lack of macro- and micronutrients, exposure to antibiotics, and a wide range of chemical and biological contaminants in food. Metabolic disorders are increasingly being treated with food products, including specialised foods containing soluble and insoluble dietary fibers [22].

In order to fight disease and promote health, we need to develop scientifically based approaches to obtaining functional ingredients with high detoxification and sorption activity.

This study aimed to develop a technology for obtaining combined dietary fibers using electrophysical methods and evaluate their detoxification properties.

## STUDY OBJECTS AND METHODS

The study objects were samples of pectin substances and combined detoxicants obtained from beet pulp.

The modified technology for obtaining pectin substances and combined detoxicants from beet pulp included the following steps:

– soaking granulated sugar beet pulp in water at 30 ± 2°C;
– hydrolysis-extraction of the pulp in an aqueous solution of succinic acid at pH 2 and 80°C for 65 min in an extremely low-frequency (29 Hz) electromagnetic field (ELF EMF) to obtain pectin extract and pulp waste;
– reaching a 5% content of pectin substances and their coagulation with 96% ethanol for 10 min;
– double washing of the coagulum in 80% ethanol;
– infrared drying of the coagulum under vacuum at 0.06 MPa and 35°C to a pectin moisture content of max. 7%;
– powdering the pectin to a particle size of max. 200 μm and double washing of the pulp waste in water;
– infrared drying of the pulp under vacuum at 0.06 MPa and 35°C to a moisture content of max. 7% to obtain cellulose;
– powdering the cellulose to a particle size of max. 200 μm; and
– further mechanical mixing of the cellulose and pectin substances at a ratio of 1:0.05 to 1:0.5.

The following samples were obtained:

– combined detoxicant obtained by extracting with succinic acid in an ELF EMF;
– combined detoxicant obtained by extracting with succinic acid;
– pectin obtained by extracting with succinic acid in an ELF EMF (without cellulose in the powder); and
– pectin obtained by extracting with succinic acid (without cellulose).

The dry powders and their profiles were identified by IR-Fourier spectrometry on an Agilent Cary 660 spectrometer. The infrared spectra were interpreted using [23].

The microstructure of the samples was determined using an XSP 10-640x microscope with a MOV-1-16x ocular micrometre.

We determined the binding capacity of dietary fibers by the trilonometric method with some modifications. In particular, 30.0 $cm^3$ of a working 0.1 M lead acetate solution was poured into a 250 $cm^3$ conical flask, to which 25.0 $cm^3$ of the model solution was added. After being held in an AVU-6s shaker, the mixture was divided into fractions with a TsLM 1–12 centrifugal apparatus. The precipitate was washed in distilled water until it had a negative qualitative reaction to lead ions

with potassium bichromate. The centrifugate and the wash waters were combined and mixed with distilled water to reach the mark in a 250 $cm^3$ flask. An aliquot of 10 $cm^3$ of the solution was titrated with 0.01 $mol/dm^3$ ethylene diamine tetraacetate (EDTA) solution at pH 9–10 using Eriochrome Black T until the colour changed from violet to blue.

The binding capacity was calculated as a metal binding percentage using the following equation:

$$BC = \frac{A_1 - A_2}{A_1} \cdot 100 \quad (1)$$

where $A_1$ is the total metal mass, g; $A_2$ is the remaining metal mass in the solution, g. $A_1$ and $A_2$ in Eq. (1) were calculated using the results of the titrometric tests as:

$$A_1 = \frac{C_{edta} \cdot V_1 \cdot M}{1000} \quad (2)$$

$$A_2 = \frac{C \cdot 250 \cdot V_{avg} \cdot M}{1000 \cdot V_2} \quad (3)$$

where $C$ is the concentration of the initial metal solution, $mol/dm^3$; $V_1$ is the initial volume of metal solution, $cm^3$; $V_2$ is the aliquot centrifugate solution taken for titration, $cm^3$; $V_{avg}$ is the average volume of EDTA taken for titration, $cm^3$; $M$ is the molecular mass of metal, Da.

## RESULTS AND DISCUSSION

Figures 1–4 show the results of identifying the profiles of the combined detoxicants and pectin substances obtained from beet pulp. The absorption bands of carbonyls in carboxyl groups were analyzed in the IR spectra of the samples.

As shown in Figs. 1–4, the absorption intensity varied in the spectral range of 800–4000 $cm^{-1}$ depending on the type of dietary fiber and the method of its preparation. The bands in the range of 100–1200 $cm^{-1}$ did not show such a dependence. The absorption bands for the valence vibrations of carboxyl group carbonyls were in the range of 1695–1710 $cm^{-1}$; those for ester groups, in the range of 1720–1740 $cm^{-1}$. Beet pectin substances had a large content of hydrolysed ester compounds, while combined detoxicants had a prevalent content of carboxyl group carbonyls, which presumably indicated their increased ability to form complexes.

We know that the complexing properties of pectin substances depend on the content of free carboxyl groups, i.e. the degree of esterification of carboxyl groups with methanol or ethanol [24, 25]. The degree of esterification determines the linear charge density of the macromolecule and, consequently, the strength

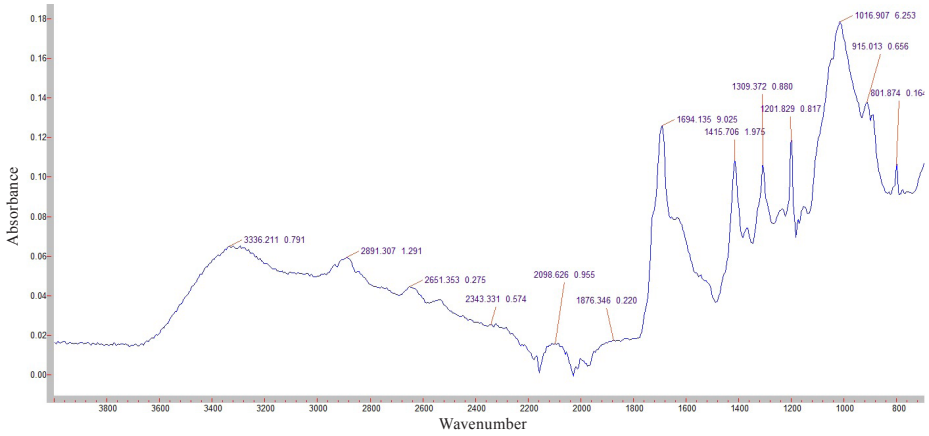



**Figure 1** IR spectrum of a combined detoxicant obtained by extracting with succinic acid in an ELF EMF

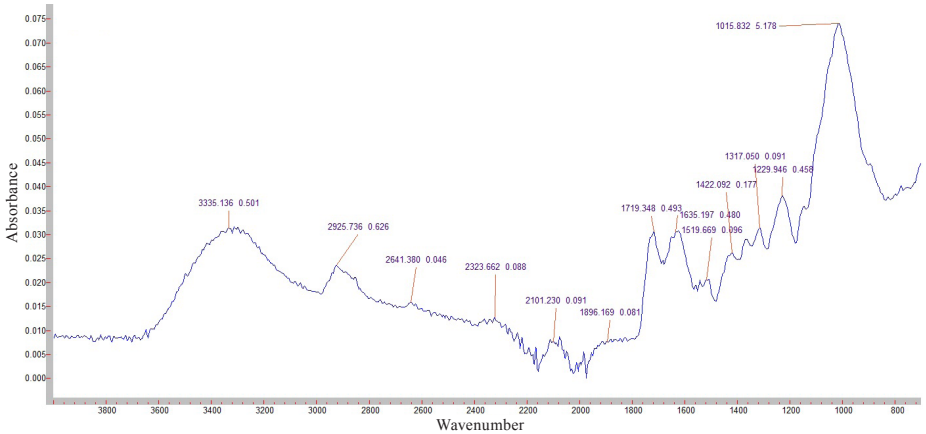



**Figure 2** IR spectrum of a combined detoxicant obtained by extracting with succinic acid

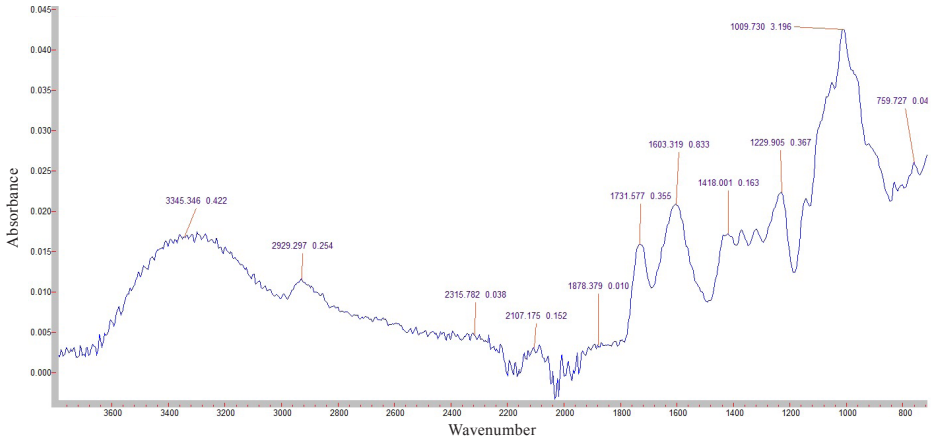



**Figure 3** IR spectrum of pectin obtained by extracting with succinic acid in an ELF EMF

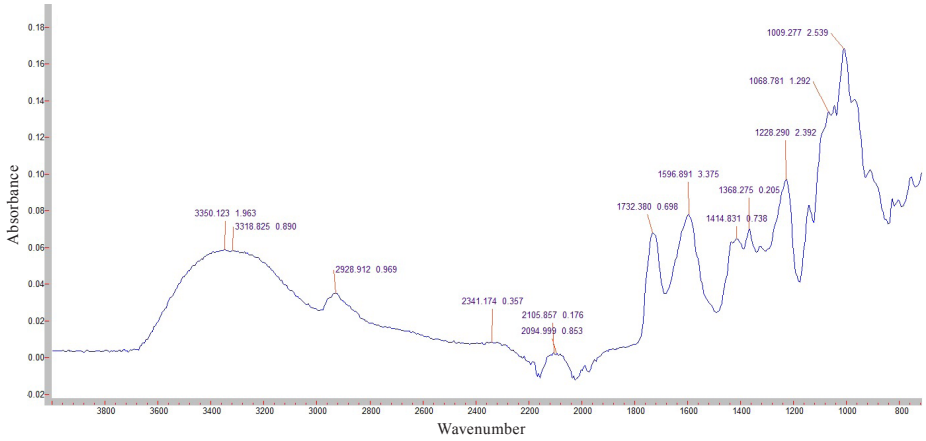



**Figure 4** IR spectrum of pectin obtained by extracting with succinic acid

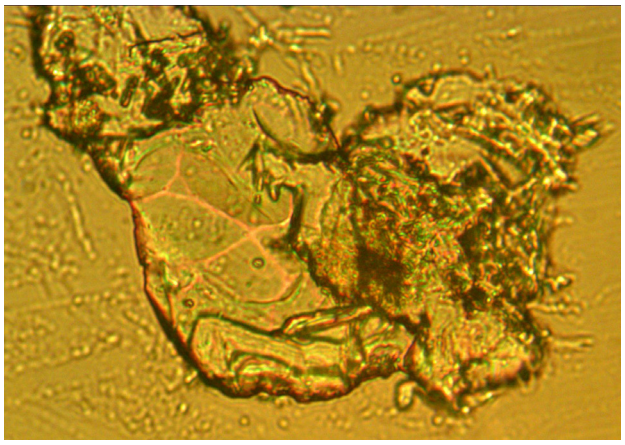


**Figure 5** Microstructure of a combined detoxicant from beet pulp (400× magnification)

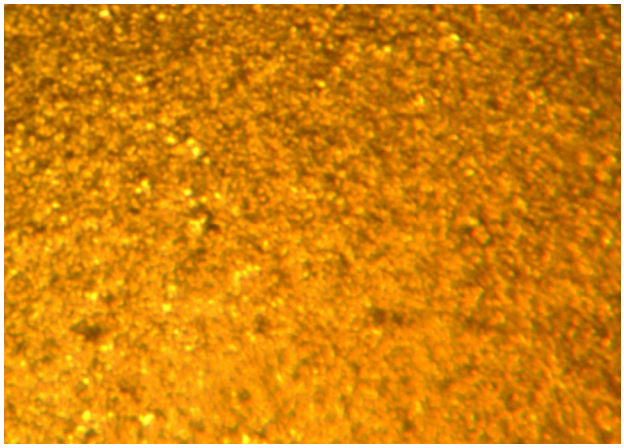


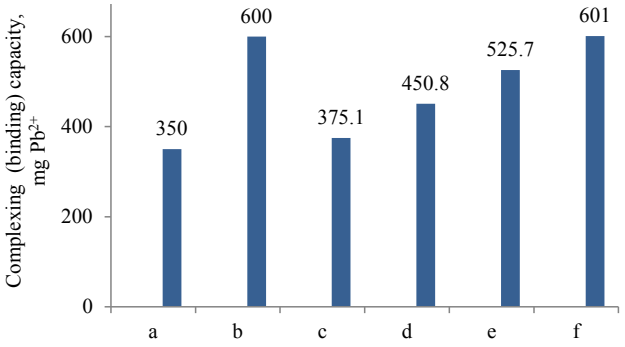
**Figure 6** Microstructure of a pectin solution (100× magnification)

and method of cations bonding [26, 27][1]. Since the detoxification activity of pectin substances is inversely proportional to its degree of esterification, we assumed that the combined detoxicants obtained through ELF EMF treatment and extraction with organic acid would have a higher binding capacity than pectin substances without cellulose.

Figure 5 shows the microstructure of the combined detoxicant obtained from beet pulp using our technology.

As we can see, the combined detoxicant had a voluminous porous structure with wide and narrow fragments and twisted zones. Unlike it, pure

[1] State Standard 29186-91. Pectin. Specifications. Moscow: Standards Publishing; 2004. 14 p.




(a) beet pulp cellulose; (b) beet pulp pectin substances; (c) cellulose and pectin substances 1:0.05; (d) cellulose and pectin substances 1:0.2; (e) cellulose and pectin substances 1:0.35; (f) cellulose and pectin substances 1:0.5

**Figure 7** The complexing (binding) capacity of a combined detoxicant with respect to lead ions ($Pb^{2+}$) depending on its qualitative composition

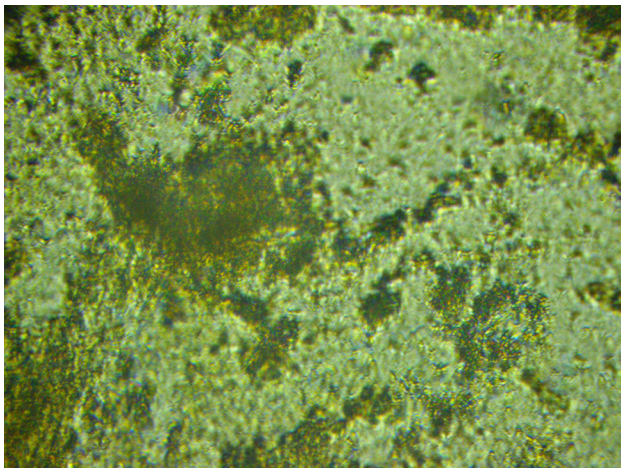


**Figure 8** Microstructure of the dispersion system during interaction between the combined detoxicant and lead acetate solution

pectin (Fig. 6) and cellulose solutions had a monotonic structure and a weakly pronounced character.

Then we studied the binding capacity of the structure-forming agents with respect to lead ions. We found that cellulose produced from beet pulp using our technology had a binding capacity of 350 mg $Pb^{2+}$/g; and pectin substances, 600 mg $Pb^{2+}$/g.

The high complexing capacity of cellulose may be due to the maceration of plant tissue under the influence of an electromagnetic field, high temperature and pH, which makes it more accessible to metabolic processes. Another factor could be the presence of residual amounts of pectin substances.

Figure 7 shows the complexing capacity of a combined detoxicant with respect to lead ions ($Pb^{2+}$) depending on its qualitative and quantitative composition.

We found that the ratio of cellulose and pectin substances from 1:0.05 to 1:0.5 provided the combined detoxicant with a good complexing capacity. The sample with a ratio of 1:0.5 showed the highest binding capacity (601 mg $Pb^{2+}$/g), clearly indicating a synergetic effect when using a combined detoxifier (an over 20% increase in binding capacity).

The increase in binding capacity could be explained by the following factors. The presence of pectin in the combined solution let to a slight decrease in the pH of the medium. As a result, it destroyed cellulose micelles and released individual macromolecules, facilitating access to free hydroxyl groups involved in complexation.

The microstructure of the dispersion system during the interaction between the combined detoxicant and lead acetate solution is shown in Fig. 8.

We assumed that the dark areas of various sizes could indicate the presence of agglomerates resulting from the complexation of lead and active groups of pectin substances and cellulose contained in the combined detoxicant.

**Table 1** Process parameters for producing combined detoxicants from beet pulp

| Process stage and parameter | Value |
|---|---|
| Soaking granulated beet pulp: | |
| temperature, °C | 30 ± 2 |
| Hydrolysis-extraction of the pulp: | |
| pH of succinic acid, units | 2 |
| temperature, °C | 80 |
| duration, min | 65 |
| EMF frequency, Hz | 29 |
| Concentrating the pulp: | |
| content of pectin substances, % | 5 |
| Coagulating pectin substances with 96% ethanol: | |
| duration, min | 10 |
| Washing the coagulum in 80% ethanol: | |
| frequency | 2 |
| Infrared drying of the coagulum: | |
| pressure, MPa | 0.06 |
| temperature, °C | 35 |
| moisture, max., % | 7 |
| Powdering the pectin: | |
| particle size, μm | 200 |
| Washing the pulp waste: | |
| frequency | 2 |
| Infrared drying of the pulp: | |
| pressure, MPa | 0.06 |
| temperature, °C | 35 |
| moisture, max., % | 7 |
| Powdering the cellulose: | |
| particle size, μm | 200 |
| Mechanical mixing of pectin and cellulose: | |
| ratio | from 1:0.05 to 1:0.5 |

Based on the experimental results, we developed a technology for producing combined detoxicants from beet pulp. The process parameters are presented in Table 1.

## CONCLUSION

As a result of the studies, we developed a technology for obtaining combined dietary fibers from beet pulp using the electrophysical method and evaluated their detoxification properties.

Identifying the profiles of the combined detoxicant samples and pectin substances obtained from beet pulp revealed that the combined detoxicants had an increased number of active groups reactive to effective complexation. A comparative evaluation of the microstructures of the combined detoxicants and pure samples of pectin and cellulose obtained from beet pulp suggested that the combined detoxicant provided a greater possibility for the chemisorption of metal ions.

The experiments established that 1:0.5 was the optimum ratio of cellulose and pectin substances in the combined detoxicant to ensure a high complexing capacity.

## CONTRIBUTION

The authors were equally involved in the writing of the manuscript and are equally responsible for any potential plagiarism.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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