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MICROWAVE AND ULTRASONIC TREATMENT AS A PRELIMINARY STAGE OF OBTAINING WATER-SOLUBLE MANNAN FROM COFFEE SLUDGE

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Introduction. Formulation of the problem

The current trend in the food industry is the use of integrated processing of raw materials which leads to an increase in the efficiency of their use. In this context it is promising to identify ways to use coffee sludge.

Currently the utilization of coffee and coffee-based beverage waste is a growing environmental problem due to its quantity [1]. In Ukraine coffee sludge is used as a raw material for pellet production, as a biofuel, as an additive to construction materials, and for the production of activated carbon. But it is not used as a source of physiologically functional ingredients. However, if coffee sludge is processed in other more modern and safe ways, it is possible to use the nutrients

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Abstract. In recent years, interest in immunocorrectors of natural origin has increased significantly. Among them is a group of polysaccharides such as mannans. Mannans are polysaccharides composed of D-mannose residues as the main chain. They possess a number of properties: immunomodulatory, cancer-protective, antimicrobial, and normalize blood cholesterol levels. Mannans are present in a number of plants, algae and microorganisms. One of the promising sources of mannans can be coffee sludge, which is accumulated on an industrial scale at instant coffee companies. This article describes a biotechnological method for the production of water-soluble mannan from coffee sludge and investigates the possibility of increasing the yield of water-soluble low-molecular-weight mannan with the maximum content of physiologically active fractions by pretreating the raw material with ultrasound and ultrahigh-frequency radiation. The sludge was pretreated in an aqueous medium using ultrasound at 25, 35 and 40 kHz for 15 minutes and treatment in a 2.45 GHz ultra-high-frequency electric field with a power of 300 W, 600 W and 800 W for 5 minutes. Next, the physical disintegrate was treated with an enzyme preparation with beta-mannanase activity and centrifuged. The resulting water-soluble products were characterized by gel chromatography to determine the molecular weight distribution of the fractions of each sample. It was found that the pretreatment with ultrasound is expedient, since the total amount of fractions with a molecular weight of less than 20 kDa is almost 80%. The treatment of raw materials with microwave rays is inefficient and leads to an increase in fractions of molecular weight less than 1 kDa. Thus, varying the conditions of the preliminary physical treatment of coffee sludge allows to regulate the molecular weight distribution of water-soluble enzymolysis products and obtain products with the required range of molecular weight values.

Key words: polysaccharides, mannan, coffee sludge, functional and physiological ingredients.

and organic compounds it contains for more productive purposes.

Analysis of recent research and publications

According to the literature [2] coffee beans contain heteromannan which contains galactopyranose units along with mannopyranosyl residues. Arabinogalactan, galactomannan and cellulose are the dominant polysaccharides in coffee beans. However in the process of producing instant coffee which is very rigorous and involves the use of high temperatures, not only does this polysaccharide depolymerize but also its individual monosaccharide residues are destroyed.

The range of applications of mannan polysaccharides in the food industry includes their use as stabilizers of suspensions and emulsions, and as structure improvers in food systems [3,4]. However the use of mannans is not limited to this area. Mannans have unique pharmacological properties. Recently some researchers have uncovered the great potential of polysaccharides contained in coffee showing that they can have tremendous functional properties. Most of these polysaccharides are not broken down by human digestive enzymes. Thus they reach the colon and potentially serve as a substrate for the colon microbiota, supporting the growth of the gut's foregut microflora: bifidobacteria and lactic acid bacteria [5-8]. In addition their therapeutic potential is associated with the presence of mannose receptors in the human body which are able to "recognize" mannose structures and interact with them in a certain way, ensuring their positive effect on the human body [9]. Coffee polysaccharides lower blood cholesterol, control blood glucose and insulin levels, and fight infectious and tumor diseases [10]. All of these biological and physicochemical properties of coffee polysaccharides can be found in the main residue obtained during the treatment of coffee powder with hot water to make instant coffee, known as spent coffee sludge which retains about 70% of the total polysaccharides present in roasted coffee [11].

Taking into account all the accumulated scientific data, there is no doubt that mannan is a promising biologically active ingredient. However, its biological activity directly depends on structural features, such as monosaccharide composition and glycosidic bonds, conformation, molecular weight, functional groups, branching and solubility [3,7,12-14].

Methods of mannans extraction depend on the nature of the raw material source. From some plant objects, where they have a reserve function, they can be extracted with water. From lignified sources where they have a structural function they are extracted by treatment with alkaline solutions. However the polysaccharide obtained in this way is insoluble in water, so acetylation and phosphorylation are more commonly performed which in turn modifies the native structure of the polysaccharide.

There is a method for extracting alkali-soluble mannan from coffee sludge. To do this first, the coffee sludge was treated with petroleum ether to degrease the raw material and then extracted with a solution of potassium hydroxide for two days. Hemicellulose was precipitated from the extract with ethanol and dried. The compounds obtained by this method are referred to as glucogalactomannans [15].

Other authors [16] isolated mannan from green defatted Arabica beans by delignification, acid saponification and subsequent alkaline extraction with a yield of 12.8%. In addition, the polysaccharides of the coffee extract were separated by alcohol precipitation and accounted for almost half of the dry

weight of the coffee extract. For the degradation of coffee mannan, both partially purified, immobilized and soluble, crude mannanase preparation were successfully used. In this way, manno-oligosaccharides including mannotetraose, mannotriose and mannobiose were obtained.

Earlier we proposed a method for obtaining modified water-soluble mannan from coffee sludge [17]. The method involves the treatment of coffee sludge with an enzyme preparation with beta-mannanase activity. The resulting polysaccharide is not native, but a modified polysaccharide, since the feedstock was subjected to a rather harsh treatment, which in some way affected the properties of its biopolymers. In addition, the treatment of the sludge with an enzyme preparation undoubtedly led to its depolymerization and changes in the monomeric composition, since no galactose residues present in the native polysaccharide were found in the target product. Thus the obtained mannan is a product of modification of the original galactomannan and differs from it in both its monomeric composition and a number of properties.

The purpose of this study is to determine the feasibility of using microwave radiation and ultrasonic treatment as a preliminary stage of coffee sludge treatment to increase the yield of water-soluble low-molecular weight fractions of mannan with a maximum content of physiologically active fractions.

To achieve this goal, the **following objectives** were solved:

- to substantiate the feasibility and determine the conditions for the use of ultrasonic and microwave treatment of coffee sludge as a preliminary stage in the development of a method for mannan extraction;
- to characterize the mannan obtained by a combined method using enzymatic and ultrasonic or microwave treatment of the raw material;
- to determine the molecular weight distribution of the obtained products to substantiate the rational conditions of ultrasonic and microwave processing.

Research materials and methods

The raw material used in the experiments was coffee sludge, a mixture of Arabica and Robusta in a ratio of 60 to 40%, obtained after the production of instant coffee at an enterprise in Odesa. The sludge was taken immediately after the extraction of water-soluble substances from it and dried to a moisture content of 7%.

An enzyme with β -endo-mannanase activity of 50,000 units was used in the study, manufactured by Heilongjiang Huatin Bio-Technology Co.

The coffee sludge was pretreated by ultrasound in an aqueous agent in ultrasonic baths with a frequency of 25, 35 and 40 kHz for 15 minutes, hydromodule 30, and microwave treatment in an ultra-high-frequency electric field with a frequency of 2.45 GHz with a power of

300 W, 600 W and 800 W for 5 minutes, hydromodule 30.

After pretreatment, the coffee sludge was subjected to enzymatic hydrolysis by β -endo-mannanase at $T = 50^\circ\text{C}$, pH 5.5, hydromodule 40 at an enzyme:substrate ratio of 1:25 for a process duration of 48 hours.

Gel chromatography was performed on Sephadex G-50 filled chromatographic columns (Pharmacia, Sweden). The column was calibrated with markers of known molecular weights. Markers: Raffinose (Pharmacia, Sweden) 504 Da; Leuconostoc mesenteroides dextran with a molecular weight of 9000–11000 Da (No. 31416 "Sigma", Germany); Leuconostoc mesenteroides dextran with a molecular weight of 15000 Da (No. 51227 "Sigma Aldrich", Germany); dextran Leuconostoc mesenteroides with a molecular weight of 25000 Da (No. 31419 "Fluka", Germany); dextran Leuconostoc mesenteroides with a molecular weight of 35000 Da (No. D1662 "Sigma", Germany); dextran Leuconostoc spp. with a molecular weight of 70000 Da (No. 31390 "Sigma Aldrich", Germany). The prepared column filled with Sephadex was injected with 7-10 mg of mannan degradation products, the eluent was water. Fractions of 2 cm³ were taken into test tubes. The carbohydrate content of the fractions was determined by the Antron method.

The experimental data were processed by means of variation statistics. The permissible value of the relative error was considered to be no more than 5%.

Results of the research and their discussion

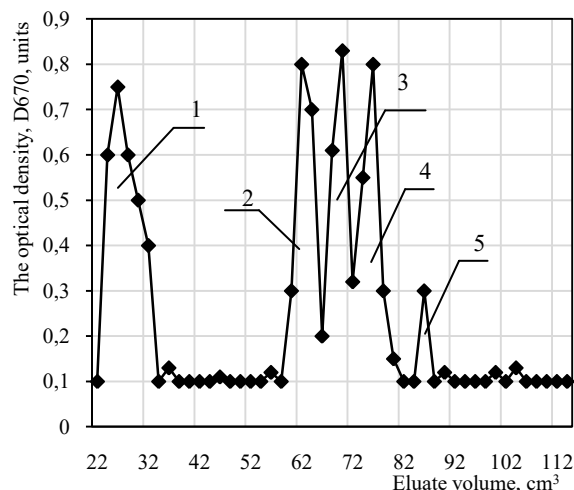
According to the literature, mannans with a molecular weight of about 20 kDa have the greatest physiological effect [9], so we established optimal conditions for obtaining water-soluble mannan with the highest content of physiologically active fractions. These conditions are the enzyme:substrate ratio of 1:25, hydromodule 40, and the hydrolysis duration of 48 hours. Gel chromatography of such a sample showed the presence of 40,5% of fractions with a molecular weight of more than 20 kDa, 50.1% with a molecular weight of 10-25 kDa and 9.4% with a molecular weight of less than 0.5 kDa (Fig. 1). At the same time, the yield of water-soluble mannan was 18.7% of the dry weight of the starting material [17].

To evaluate the possibility of reducing the strength of intermolecular bonds of biopolymer components of coffee sludge and, accordingly, increasing the accessibility of hemicelluloses to the enzyme, we tried to apply pretreatment of raw materials with ultrasound and microwave.

The application of ultrasonic vibrations in the extraction of bioactive substances is very promising. In many cases, it provides an exceptionally high intensity of the technological process which is unattainable with such common methods as mechanical stirring, application of high temperatures and pressures, etc.

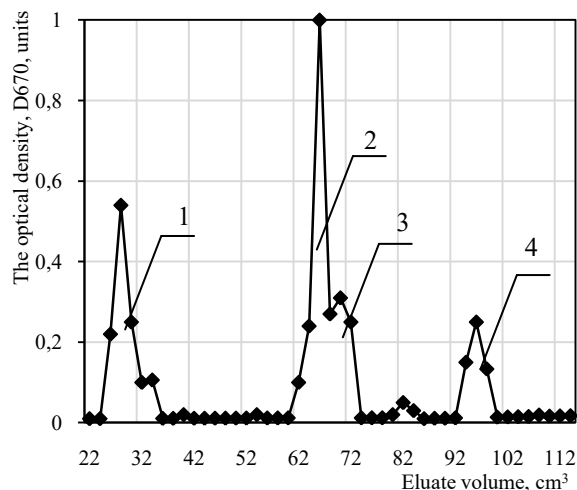
Previously, it was believed that high-frequency oscillations (at least 300–500 kHz) were required to

intensify technological processes. Recently, acoustic vibrations of both medium and low intensity have been successfully used [18]. Therefore, to increase the accessibility of coffee sludge hemicelluloses to the enzyme, we tried to apply pretreatment of raw materials with ultrasound at frequencies of 25, 35 and 40 kHz for 15 minutes, hydromodule 30. After which, the enzymatic hydrolysis of the mannan component was carried out by β -endo-mannanase with an activity of 50,000 units/g at $T = 50^\circ\text{C}$, pH 5.5, hydromodule 40 at an enzyme:substrate ratio of 1:25 for a process duration of 48 hours and the distribution of the molecular weight of the resulting hydrolysates was studied (Fig. 2-4).



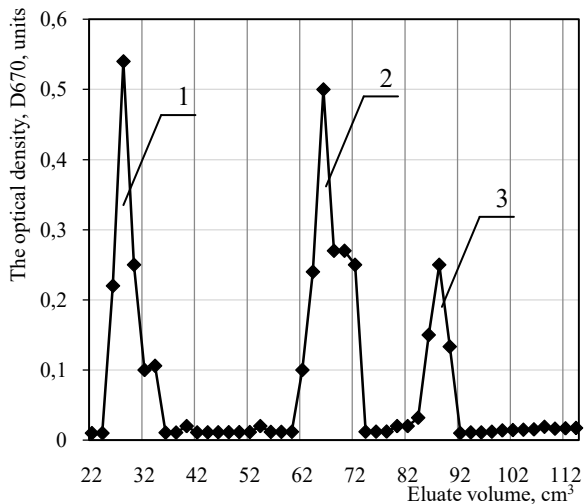
- 1 - fragments with molecular weights of more than 30 kDa;
- 2 - fragments with a molecular weight of 25 kDa;
- 3 - fragments with a molecular weight of 20 kDa;
- 4 - fragments with a molecular weight about 10 kDa;
- 5 - fragments with a molecular weight less than 1 kDa

Fig. 1. Gel chromatogram of water-soluble mannan



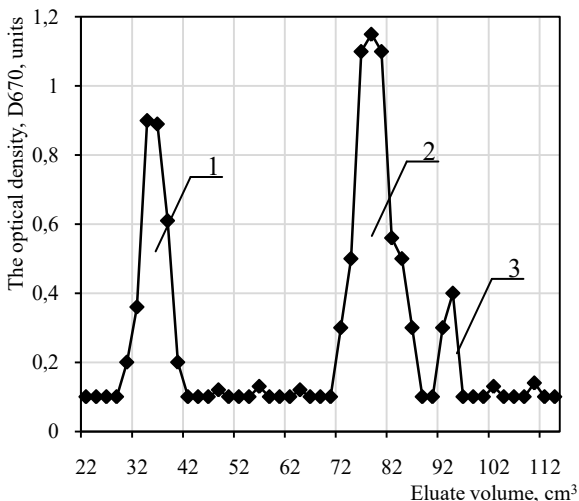
- 1 - fragments with molecular weights of more than 30 kDa;
- 2 - fragments with a molecular weight of 25 kDa;
- 3 - fragments with a molecular weight of 20 kDa,
- 4 - fragments with a molecular weight less than 1 kDa

Fig. 2. Gel chromatogram of water-soluble mannan which was pretreated with ultrasound at a frequency of 25 kHz



1 – fragments with molecular weights greater than 30 kDa;
2 – fragments with molecular weights of 20 kDa;
3 – fragments with a molecular weight less than 1 kDa

Fig. 3. Gel chromatogram of water-soluble mannan which was pretreated with ultrasound at a frequency of 35 kHz



1 – fragments with molecular weights greater than 30 kDa;
2 – fragments with molecular weights of 10 kDa;
3 – fragments with a molecular weight less than 1 kDa

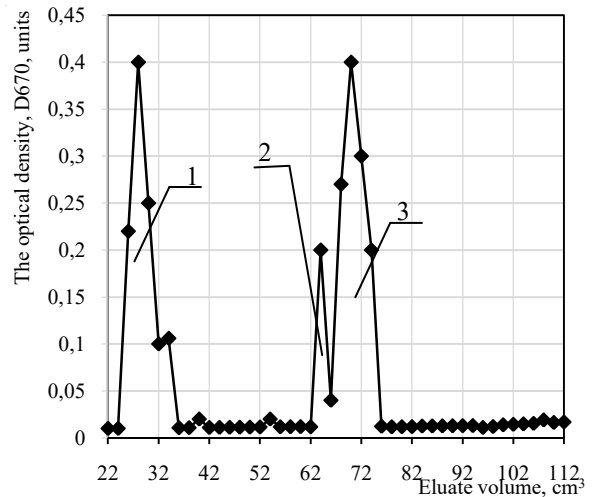
Fig. 4. Gel chromatogram of water-soluble mannan which was pretreated with ultrasound at a frequency of 40 kHz

The yield of water-soluble mannan ranged from 19.1–20.4% of the dry weight of the feedstock, depending on the conditions of sonication.

It is known [19] that microwave treatment is an effective factor contributing to the destruction of cellular structure elements due to their dielectric destruction. In this regard, we considered the possibility of its use in the preparation of water-soluble mannan as an alternative to other methods.

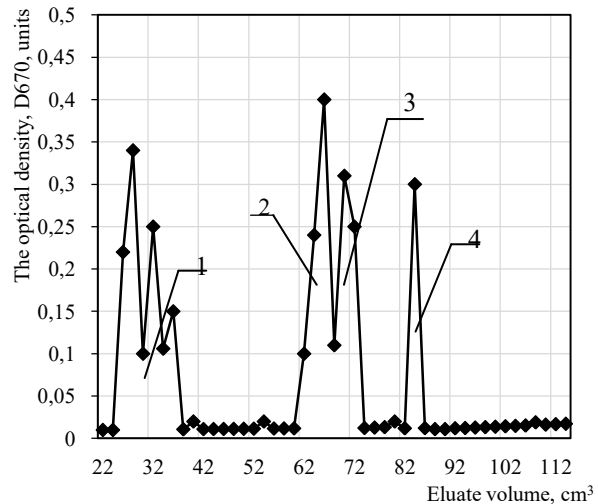
The microwave treatment was carried out with microwave beams in an ultra-high-frequency electric field of 2.45 GHz. In the studies, the power was varied at 300 W, 600 W, and 800 W for 5 minutes. After treatment, enzymatic hydrolysis was performed under the same conditions. Gel chromatograms illustrating

the molecular weight distribution of enzyme hydrolysis products with microwave treatment are shown in Figures 5-7. The yield of water-soluble products did not change compared to the preparation obtained without pretreatment and ranged from 18.1–18.9% of the dry weight of the starting material, depending on the treatment conditions.



1 – fragments with molecular weights of more than 30 kDa;
2 – fragments with molecular weights of 20 kDa;
3 – fragments with molecular weights of 10...15 kDa

Fig. 5. Gel chromatogram of water-soluble mannan pretreated with 300 W microwave beams



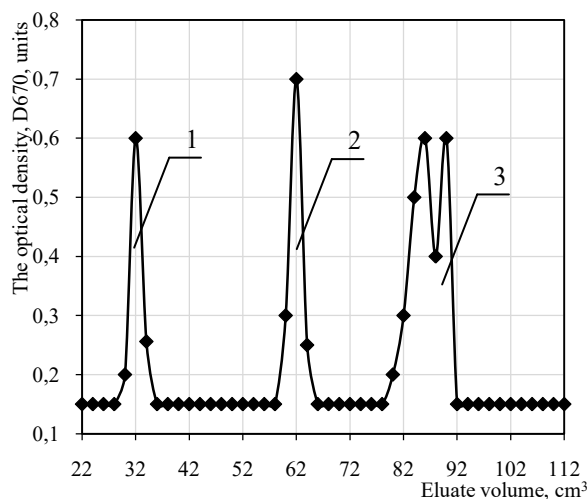
1 – fragments with molecular weights greater than 30 kDa;
2 – fragments with molecular weights of 20 kDa;
3 – fragments with molecular weights of 10 kDa;
4 – fragments with a molecular weight less than 1 kDa

Fig. 6. Gel chromatogram of water-soluble mannan pretreated with 600 W microwave beams

The data obtained for determining the molecular weight distribution of mannan samples are summarised in Table 1. As can be seen from the results presented (Table 1), the preliminary ultrasonic treatment of coffee sludge in an aqueous medium leads to a change in the distribution of molecular weights of the

enzymolysate, compared to those obtained without prior physical impact, which is reflected in the profiles of gel chromatograms of the samples (Fig. 1).

The treatment of raw materials with ultrasound at a frequency of 25 kHz (Fig. 1) leads to a decrease in the number of mannan fractions with a molecular weight of about 20 kDa, while the number of fragments with a molecular weight of less than 1 kDa increases. The same trend is observed when sonicated at a frequency of 35 kHz (Fig. 3).



1 - fragments with molecular weights greater than 30 kDa;

2 - fragments with molecular weights of 20 kDa;

3 - fragments with molecular weights less than 1 kDa

Fig. 7. Gel chromatogram of water-soluble mannan pretreated with 800 W microwave beams

Table 1 – Molecular weight distribution of water-soluble mannan fractions depending on the conditions of raw material pretreatment

| Sample | Molecular weight of the fractions, %. | | | Water-soluble product yield, % w/w |
|-----------------------------|---------------------------------------|-----------|-----------------|------------------------------------|
| | over 30 kDa | 10–25 kDa | less than 1 kDa | |
| Initial values | 40.5 | 50.1 | 9.4 | 18.7 |
| Ultrasonic treatment | | | | |
| 25 kHz | 31.5 | 47.8 | 20.7 | 19.1 |
| 35 kHz | 37.1 | 51.5 | 11.4 | 19.7 |
| 40 kHz | 11.9 | 78.1 | 10.0 | 20.4 |
| Microwave treatment | | | | |
| 300 W | 41.6 | 58.4 | - | 18.4 |
| 600 W | 21.3 | 36.7 | 42.0 | 18.1 |
| 800 W | 15.7 | 19.7 | 64.6 | 18.9 |

However, the pretreatment of coffee sludge with ultrasound at a frequency of 40 kHz (Fig. 4), on the contrary reduces the yield of fractions of enzymolysate with a molecular weight of more than 20 kDa by almost 3 times and amounts to 11.9%, while the content of target fractions with a molecular weight of 10–20 kDa increases by 78.1%, and fractions with a molecular weight of less than 1 kDa by 11.4%. Thus pretreatment with ultrasound is advisable. In addition, the yield of water-soluble products increases, and the total amount of fractions with a molecular weight of less than 20 kDa is almost 90%.

The results of the study on the pretreatment of coffee sludge with microwave with a power of 300 to 800 W showed that these exposure parameters do not lead to the production of mannan fractions with the target molecular weight. When coffee sludge was pretreated with 300 W of microwave radiation, the content of fractions with a molecular weight of more than 30 kDa was 41.6%, fractions with a molecular weight of 10 to 25 kDa were 58.4%, and fractions with a molecular weight of less than 1 kDa were absent. When the power is increased to 600 W, there is a marked decrease in the target fraction to 36.7%, and the fraction with a molecular weight of less than 1 kDa is 42.0%. The same situation occurs when coffee sludge is treated with microwave radiation at a power of 800 W, the yield of fractions with a molecular weight of more than 30 kDa is 15.7%, fractions with a molecular weight of about 10–25 kDa – 19.7%, and fractions with a molecular weight of less than 1 kDa – 64.6%.

Conclusion

Thus the pretreatment of coffee sludge with microwave rays does not lead to an increase in the yield of water-soluble products, nor to an increase in the fractions with the most physiologically active molecular weight, and therefore is inappropriate. However ultrasonic treatment is more promising as a pretreatment of raw materials, the purpose of which is to increase the yield of water-soluble destructed mannan with the target molecular weight, since the total amount of fractions with a molecular weight of 1–20 kDa is about 90%, while the yield of water-soluble products is 20.4% of the dry weight of the raw material.

Thus, by varying the conditions of pretreatment of coffee sludge, it is possible to "control" the molecular weight distribution of the resulting water-soluble products to some extent and move the process towards the formation of products with the desired range of molecular weights.

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НАДВИСОКОЧАСТОТНА ТА УЛЬТРАЗВУКОВА ОБРОБКА ЯК ПОПЕРЕДНІЙ ЕТАП ОТРИМАННЯ ВОДОРозчинного МАНАНУ КАВОВОГО ШЛАМУ

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Анотація. В останні роки значно зріс інтерес до імунокоректорів природного походження. Серед них можна виокремити таку групу полісахаридів, як манани – це полісахариди, основний ланцюг яких складається із залишків D-манози. Вони володіють низкою властивостей: імуномодельовальною, онкопротекторною, антимікробною, нормалізують рівень холестерину в крові. Манани присутні у ряді рослин, водоростях та мікроорганізмах. Одним з перспективних джерел отримання мананів може бути кавовий шлам, який у промислових масштабах накопичується на підприємствах, що виробляють розчинну каву. У даній статі розглянуто біотехнологічний спосіб отримання водорозчинного манану з кавового шламу та досліджено можливість збільшення виходу водорозчинного низькомолекулярного манану з максимальним вмістом фізіологічно активних фракцій завдяки попередній обробці сировини ультразвуком та надвисокочастотним випромінюванням. Попередню обробку шламу проводили у водному середовищі, використовуючи ультразвук частотою 25, 35 та 40 кГц протягом 15 хвилин та обробку в надвисокочастотному електричному полі з частотою 2,45 ГГц потужністю 300 Вт, 600 Вт та 800 Вт впродовж 5 хв. Далі дезінтегрували, отриманий шляхом фізичного впливу, обробляли ферментним препаратом з бета-мананазною активністю та центрифугували. Отримані водорозчинні продукти характеризували методом гель-хроматографії для визначення молекулярно-масового розподілу фракцій кожного зі зразків. Встановлено, що попередня обробка ультразвуком є доцільною, оскільки сумарна кількість фракцій з молекулярною масою меншою 20 кДа складає практично 80%. Обробка сировини променями НВЧ є неефективною і приводить до збільшення фракцій молекулярної маси менше 1 кДа. Таким чином, варіювання умов передньої фізичної обробки кавового шламу дозволяє регулювати молекулярно-масовий розподіл водорозчинних продуктів ферментолізу та отримувати продукти з бажаним діапазоном значень молекулярних мас.

Ключові слова: полісахариди, манан, кавовий шлам, функціонально-фізіологічні інгредієнти.