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Розроблено технологію отримання дієтичної добавки антиліполітичної дії. Інгібітор ліпази – фенольні сполуки ріпаку іммобілізовано на біополімерному комплексі гливи звичайної. Показано, що іммобілізована форма інгібітору у порівнянні з інтактною має кращі фізико-хімічні показники, а також ступінь збереження активності як в умовах шлунково-кишкового тракту, так і при зберіганні

Ключові слова: технологія, дієтична добавка, інгібітор ліпази, біополімерний комплекс, гриби, ріпак

Разработана технология получения диетической добавки антилиполитического действия. Ингибитор липазы – фенольные соединения рапса иммобилизированы на биополимерном комплексе вешенки обыкновенной. Показано, что иммобилизованная форма ингибитора по сравнению с интактной имеет более высокие физико-химические показатели, а также степень сохранения активности как в условиях желудочно-кишечного тракта, так и при хранении

Ключевые слова: технология, диетическая добавка, ингибитор липазы, биополимерный комплекс, грибы, рапс

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A TECHNOLOGY OF AN ANTILIPOLYTIC DIETARY SUPPLEMENT BASED ON PHENOLIC COMPOUNDS AND BIOPOLYMERS OF PLEUROTUS OSTREATUS

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1. Introduction

The analysis of many studies revealed a cause-and-consequence relationship between obesity and diseases such as insulin-independent diabetes, hypertension, atherosclerosis, gallstone disease, and some malignancies [1, 2]. In 1998, the World Health Organization (WHO) declared obesity an independent chronic disease that without proper treatment can lead to disorders of the cardiovascular, digestive, respiratory and endocrine systems [1, 3].

Today, obesity is found in 300 million people, and about 1.7 billion people are overweight. The number of people with this disorder is growing in all countries regardless of their level of economic development. According to experts, if the existing tendency of growth continues, already in 2025 about 40 % of men and 50 % of women will suffer from obesity.

The total economic costs of treating obesity and its consequences exceed those of cancer [1, 4]. Thus, obesity is acquiring the status of a national problem. In order to preserve the health of the nation, which is the main resource of the state development, a search for new effective means of correcting weight becomes an urgent contemporary task.

Thus, the relevance of the work in this direction is determined by the fact that today the range of medicines to combat obesity is very limited and not produced in Ukraine at all. Therefore, it is advisable to develop weight-correction remedies on the basis of a regional various-source plant material that would provide multi-effect treatment of high safety and at low cost.

2. Analysis of the previous studies and statement of the problem

Being overweight is the result of a positive balance between the amount of energy that a human body generates from food during the day and the amount of energy that a person spends on vital and physical activities. Weight loss occurs only if there is energy deficit in the body, which can be achieved by reducing food intake or increasing energy outflow. Therefore, the main method of weight loss is keeping to a hypocalory diet while increasing physical activity [5, 6].

Unfortunately, clinical research proves that the above method alone often fails to lead to the desired result, which

is predetermined by several factors. Firstly, the modern pace of life complicates keeping to a diet, i. e. taking food 4–5 times a day. Secondly, an obesity-preventive diet is based on foodstuffs with a high content of fibre, vitamins and other biologically active substances such as cereals, whole grains, fruits, and vegetables [5]. However, modern technologies of processing and, to some extent, cultivating raw materials reduce the amounts of these components in food and increase the mass fraction of easily digestible carbohydrates, which adds calories to a daily diet [7]. Thirdly, after some weight loss at the stage of the parameter stabilization, most people take a passive position and, consequently, restore the original weight or even exceed it [8]. To enhance the efficiency of the main methods of weight loss, prevent recovery of weight and improve metabolic parameters, it is necessary to use remedies that are capable of adjusting the body weight [9–12].

Initially there were a significant number of medicines in this group, with various mechanisms of action [9], although further research revealed their serious side effects and reduced the list of recommended preparations to two [10–12]. The mechanism of action of one of them – sibutramine – consists in suppressing the appetite due to an effect on neurotransmitter metabolism. However, after almost thirteen-year experience of its use, it was also withdrawn from the market because of a number of side effects on the cardiovascular system [10].

At present, the only licit drug that is used to adjust body weight is orlistat. It is a semi-synthetic medicine of non-Ukrainian production that is hydrogenated from lipstatine – a metabolic product of the microorganism *Streptomyces toxytricini*. Orlistat inhibits the action of gastric and pancreatic lipases, i. e. it has an antilipolytic effect, inhibits the hydrolysis of fats, and reduces their absorption in the body [11, 12]. However, this preparation is not devoid of side effects, the most serious of which include provoking significant changes in qualitative and quantitative compositions of intestinal microflora and further disrupting of the entire microbial-tissue complex [13].

An effect similar to that of orlistat is displayed by phenolic compounds of rapeseed, which are characterized by low allergy potential as well as lack of toxicity and addictive effect. Their only downside is that in the acidic environment of the stomach they almost completely lose their inhibitory activity [14].

To meet the demand for high-safety antilipolytic medicines capable of weight adjustment, it is important to develop a technology of obtaining an antilipolytic dietary supplement from compounds of plant origin.

3. The aim and objectives of the research

The aim of our research was to develop a technology of obtaining an antilipolytic dietary supplement on the basis of substances derived from plant materials – phenolic compounds of rapeseed and a biopolymer complex of oyster mushrooms.

The aim can be achieved through the following basic objectives of the research:

– to study the effect of conditions of the matrix – a biopolymer complex of oyster mushrooms and parameters for immobilizing phenolic compounds of rapeseed – on antilipolytic activity of the samples;

– to provide physical and chemical characteristics of the resulting products and to determine any change in the antilipolytic activity of medicines during their storage;

– to develop rational modes of the technology of obtaining an antilipolytic dietary supplement;

– to specify the chemical structure and the quality parameters of the new dietary supplement.

4. Materials and methods of studying the effect of conditions under which immobilized preparations are obtained on the antilipolytic, physical and chemical parameters thereof

4. 1. Test materials and equipment for the experiment

The experimental study was based on samples of a biopolymer complex of oyster mushrooms – solid residues obtained by sequential processing of raw mushrooms with hot water, a 3.7 % solution of HCl at room-temperature as well as a 3.0 % or a 7.0 % solution of NaOH at +98 °C. The treatment lasted for 90 min and 270 min.

The lipase inhibitor, which was represented by phenolic compounds of rapeseed, was extracted by applying a 96 % ethanol to pre-crumbled defatted raw material. The mixture was centrifuged, the solvent was removed from the extract by means of a rotary vacuum evaporator, and the residue was dissolved in water to obtain a solution of a certain concentration of the inhibitor.

Immobilization was performed through saturation of the biopolymer complex with a solution of phenolic compounds of rapeseed. The mass fraction of the inhibitor in the preparations (in terms of dry matter) was modified within the range of 0.5–8.0 %, the immobilization temperature ranged from +20 °C to +40 °C, the hydrological module (HM) was 2–10, and the immobilization lasted from 10 to 50 min. The resulting product was dried.

The IR spectra of the samples were recorded on the Fourier IR-spectrometer FTIR-8400S in the range of 4000–400 cm^{-1}. A quantitative differential analysis of the resulting IR-spectra was performed according to [15].

4. 2. Methods to determine the chemical structure of the samples and their physical and chemical parameters

The chemical structure of the samples of the biopolymer complex of oyster mushrooms and the antilipolytic dietary supplement were determined by means of their hydrolyzation with solutions of mineral acids [16] and subsequently studied for specifying the monosaccharide structure of hydrolysates by using the chromatograph Hewlett Packard 5890 [17]. Glucose was determined with the help of anthrone [18], with glucose (*Sigma-Aldrich Corporation*, USA) taken as the standard; glucosamine was determined by the spectrophotometric method [19], with glucosamine (*Sigma-Aldrich Corporation*, USA) taken as the standard; melanin was determined by the spectrophotometric method [20], with melanin (*Sigma-Aldrich Corporation*, USA) taken as the standard. Protein nitrogen was calculated as the difference between total nitrogen, which was determined by the Kjeldahl method according to the DSTU ISO 8968-1:2005 (IDF 20-1:2001), and chitin nitrogen, which was calculated by dividing the chitin content by factor 6.89 [21]. Total protein was calculated by multiplying the protein nitrogen by 6.25. The content of phenolic compounds in the solution of the inhibitor was determined by the Folin-Denis method [22].

The antilipolytic activity of the samples was calculated as the difference between the value of lipolytic activity, which was determined in accordance with [23], intact lipase and lipase in the presence of an inhibitor solution or an inhibitor that was immobilized by the biopolymer complex of oyster mushrooms. The antylipolytic activity of the inhibitor was accepted as 100 % activity, whereas the immobilized form activity was calculated as a percentage of this value.

To determine the pH stability, the samples were incubated in solutions with a pH value of 2.0–9.0 for 0–360 min, which was followed by bringing the pH solution to 7.0 and determining the antilipolytic activity. Thermostability of the products was assessed by their incubation at (+20±2) °C, (+37±2) °C and (+65±2) °C for 0–360 min, which was followed by bringing the temperature to (+37±2) °C and determining the antilipolytic activity.

To simulate the behaviour of the immobilized preparation in the gastrointestinal tract, a number of samples were consistently incubated in gastric juice (for 180 min) and natural bile (for 180 min) at (+37±2) °C. Every 60 minutes, a sample was selected, the pH solution was adjusted to 7.0, and thus we measured the degree of antilipolytic activity preservation.

The water-holding capacity (WHC) and fat-binding properties (FBP) of the dietary supplement were determined by [24], the lead ion sorption by [24], the cholic acid sorption by [243], the phenol sorption by [24], the antioxidant activity by [24], and the growth stimulation effect on lactobacilli and bifidobacteria by [24]. The qualitative and quantitative structures of the microbiota were assessed by determining the number of E. coli bacteria (coliform bacteria) in accordance with the GOST 30518-97, whereas mesophilic aerobic and facultative anaerobic bacteria were counted according to the DSTU IDF 100B:2003.

6. The research findings on the effect that conditions of obtaining immobilized preparations produce on their antilipoliytic activity and physical and chemical parameters

The lipase inhibitor was immobilized with the help of a sample series of a biopolymer complex of oyster mushrooms that was used as the matrix. The data presented in Table 1 show that the samples have a high content of polysaccharides. The latter, according to the analysis of the monosaccharide structure of their hydrolysates, are represented by glucan and chitin. Biopolymer complexes of oyster mushrooms also contain melanins and protein.

Table 1

The structure of the samples of biopolymer complexes

Sample number	Conditions of obtaining		Content of components (%)			
	Concentration of NaOH solution (%)	Duration of exposure (min)	Glucan	Chitin	Protein	Melanins
1	3	90	81.3	7.5	3.5	2.5
2		270	78.6	8.1	3.0	3.4
3	7	90	73.9	10.0	3.8	7.9
4		270	66.4	12.7	3.2	10.3

Immobilization of the lipase inhibitor on biopolymer complexes 1–4 resulted in immobilized-inhibitor preparations marked as 1'–4', respectively.

The study has disclosed the effect of the mass fraction of rapeseed phenolic compounds in the resulting products' structure on their antilipolytic activity, which showed that when the inhibitor content in the samples was increased from 0.5 % to 1.0 %, it improved their antilipolytic activity (Table 2). A further increase in the number of phenolic compounds did not lead to significant changes. Maximum antilipolitic activity was revealed in the samples in which the matrix was represented by biopolymer complexes of oyster mushrooms obtained by processing the raw material with a 7 % solution of alkaline agent.

To determine the rational conditions of immobilization, we studied the effect of the hydrological module, the temperature and the process duration produced on the level of antilipolytic activity of the derived preparations.

Table 2

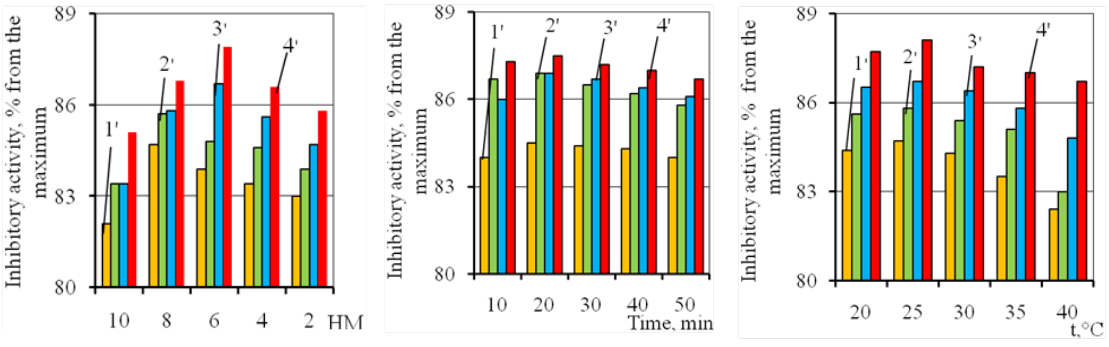
Antilipolytic activity of the inhibitor immobilized on the samples of the biopolymer complex

Mass fraction of the inhibitor (%)	Antilipolytic activity of the samples (% from the maximum)			
	№ 1'	№ 2'	№ 3'	№ 4'
8.0	84.8	85.8	88.0	88.2
6.0	84.9	85.9	87.8	88.1
4.0	84.7	85.7	87.4	87.6
2.0	84.6	85.6	87.5	87.9
1.0	84.4	85.4	86.8	87.3
0.7	79.2	80.2	82.1	82.4
0.5	66.8	68.8	72.9	73.5

It has been determined that preparations derived with the help of a 3 % and a 7 % alkali solution have hydrological module values equal to 8 and 6, respectively (Fig. 1, *a*).

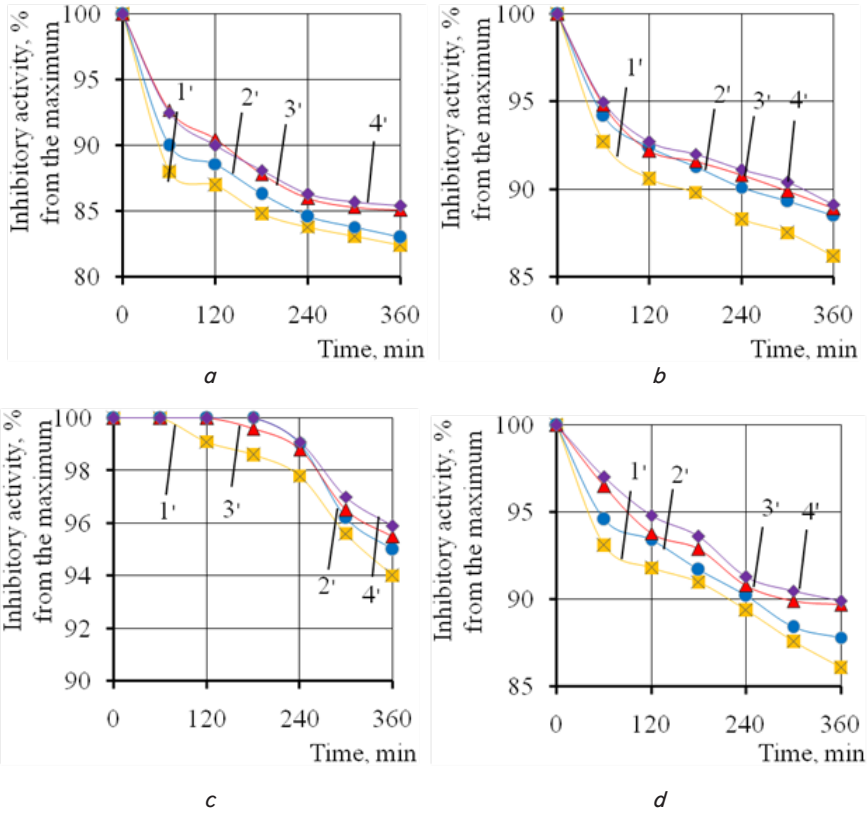
The results of studying the effects of temperature and process duration on the inhibitory activity of the samples is shown in Fig. 1, *b, c*. According to the data, the appropriate temperature and time for immobilization are +20–25 °C and 20 minutes, respectively.

The analysis of the comparative characteristics of the pH- and thermal stability of the immobilized and free forms of the inhibitor shows that immobilization enhances the inhibitor stability under changing environmental conditions. Thus, antilipolytic activity of immobilized phenolic compounds depends on the conditions of obtaining a biopolymer complex: exposure to 360 min of incubation at pH=2.0 results in 82.4–85.1 % of the initial value (Fig. 2). Under these conditions, intact inhibitor retains only 40.0 % of its initial activity. Subjecting samples to higher pH values of the environment is practically not accompanied by changes in the value of this parameter. Exposure of an intact lipase inhibitor during 360 minutes at +65 °C leads to a loss of 70 % of the initial activity, whereas exposure of an immobilized inhibitor results in a loss of only 20.6-24.2 % (Fig. 3). The most stable products are those where the carriers are samples of biopolymer complexes No. 3 and No. 4.



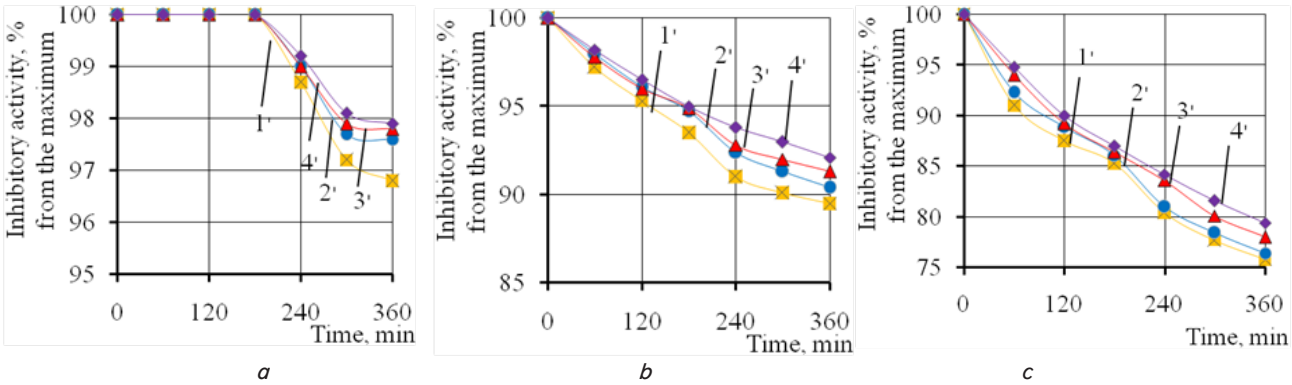
a *b* *c*

Fig. 1. The effect of immobilization conditions on the antilipolytic activity of immobilized inhibitors: *a* – hydrological module, *b* – time, *c* – temperature: 1' – sample No. 1'; 2' – sample No. 2'; 3' – sample No. 3'; 4' – sample No. 4'



a *b* *c* *d*

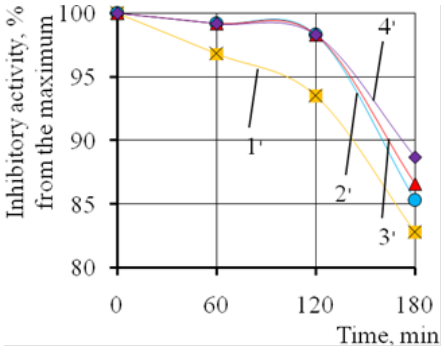
Fig. 2. pH-stability of immobilized preparations: *a* – pH=2.0; *b* – pH=5.0; *c* – pH=7.0; *d* – pH=9.0: 1' '– sample No. 1'; 2 – sample No. 2'; 3' – sample No. 3'; 4' – sample No. 4'



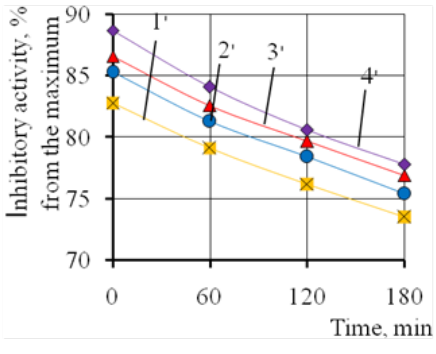
a *b* *c*

Fig. 3. Thermal stability of immobilized preparations: *a* – t=(+20±2) °C; *b* – t=(+37±2) °C; *c* – t=(+65±2) °C: 1' – sample No. 1'; 2' – sample No. 2'; 3' – sample No. 3'; 4' – sample No. 4'

Since many biologically active substances lose their activity after exposure to the aggressive environment of the gastrointestinal tract, we modelled the behaviour of immobilized preparations in digestion. It has been found that after incubation in a gastric juice an immobilized inhibitor preserves most of its antilipolytic activity (Fig. 4). Further exposure of the immobilized inhibitor to bile reduces the value to 73.5–77.8 %, whereas the intact inhibitor retains only 40.4 % of its antilipolytic activity under the same conditions.



a



b

Fig. 4. Changes in the activity of immobilized preparations in conditions that simulate normal digestion: *a* — gastric juice; *b* — bile: 1' — sample No. 1'; 2' — sample No. 2'; 3' — sample No. 3'; 4' — sample No. 4'

The data (Fig. 5) prove that antilipolytic activity is more robust in the preparations in which the carrier is represented by the samples of a biopolymer complex of oyster mushrooms extracted by treating the raw material with a more concentrated solution of NaOH (No. 3 and No. 4).

The nature of interaction between the inhibitor and the carrier was determined through the IR-spectroscopy. Thus, sample No. 3' shows that in the differential IR-spectrum of correlating a mechanical mixture of the carrier and the inhibitor absorption bands of 1648–1690 cm^{-1} and 2900–3400 cm^{-1} become less intensive. This indicates that an inhibitor immobilization consists in interaction between hydroxyl inhibitor groups and the matrix, i. e. the inhibitor-matrix complex is formed through a system of hydrogen bonds.

Thus, the analysis of the obtained data leads to the conclusion that the rational conditions of immobilization include applying the solution of a 0.17 % lipase inhibitor with HM 6 to a biopolymer complex of oyster mushrooms, at +20–25 °C and with immobilization time of 20 minutes. Biopolymer complex No. 3 should be used as a matrix.

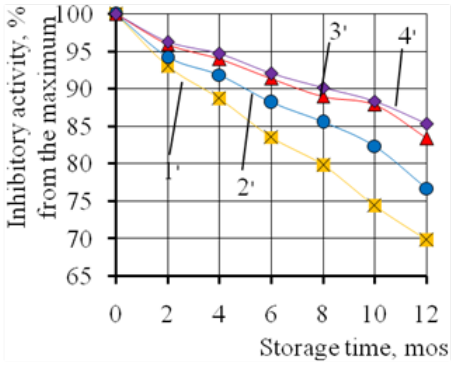


Fig. 5. Changes in the inhibitory activity of preparations in storage: 1' — sample No. 1'; 2 — sample No. 2'; 3' — sample No. 3'; 4' — sample No. 4'

We used the determined parameters of the process to design a technological scheme of obtaining an antilipolytic dietary supplement (Fig. 6).

The chemical structure, functional and physiological properties as well as microbiological parameters of quality of the resulting product are shown in Table 3.

Table 3

The properties of the dietary supplement of antilipolytic effect

Name of the element	Value
Mass fraction of glucan, %	71.1
Mass fraction of chitin, %	9.6
Mass fraction of melanin, %	7.6
Mass fraction of protein, %	3.7
Mass fraction of phenolic compounds of rapeseed, %	1.0
Antilipolytic activity, IO/g of the product	165.2
Antioxidant activity, %	90.3
Number of bifidobacteria, 10^{12} CFU/sm^3	2.0
Number of lactobacilli,·10^8 CFU/sm^3	2.8
Cholic acid sorption, mg/g of the product	7.7
Lead sorption, mg/g of the product	7.9
Phenol sorption, mg/g of the product	5.7
Water-retaining ability, g/g of the product	6.3
Fat-binding ability, g/g of the product	2.1
MAFAM,·10^2 CFU/g	0.3
Coliform bacteria, CFU/g	absent

We have determined that the developed dietary supplement is characterized not only by its antilipolytic activity but also by high sorption and antioxidant effect; it is able to stimulate the growth of lactobacteria and bifidobacteria. Moreover, the overall effectiveness of the medicine is significantly increased by the structural presence of dietary fibres that are able to correct the body weight as they increase the feeling of satiety, facilitate the gastric emptying rate, and improve metabolic exchange. The dietary supplement is microbiologically safe and benign within 12 months of storage.

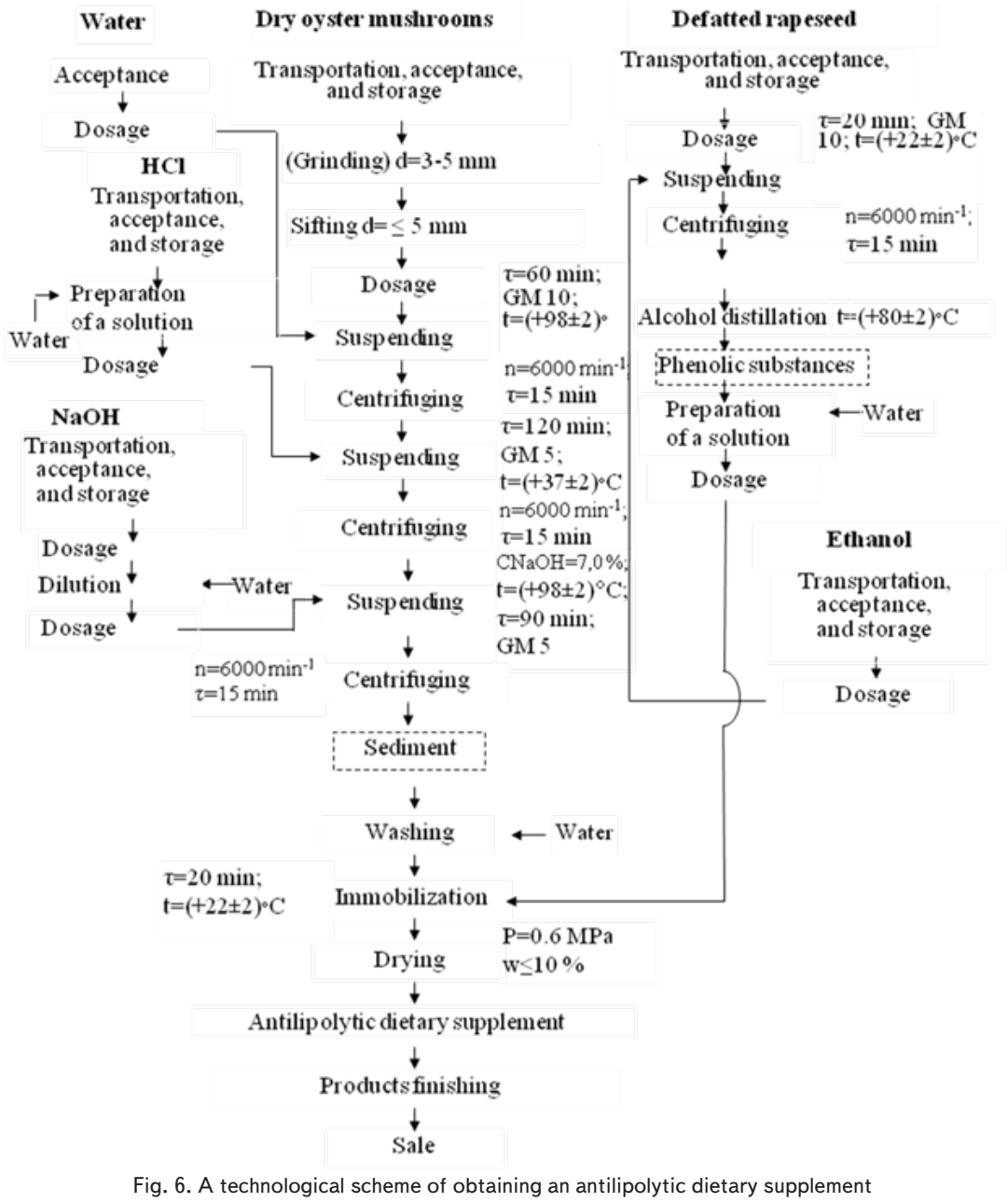


Fig. 6. A technological scheme of obtaining an antilipolytic dietary supplement

6. Discussion of the findings on determining the rational conditions for obtaining a preparation of antilipolytic effect

The data analysis has proved the expediency of immobilizing phenolic compounds of rapeseed in samples of a biopolymer complex of oyster mushrooms. All developed preparations were found to have a higher thermal stability than the free inhibitor (Fig. 3). This intensifies the process of drying to obtain them. Compared with the intact inhibitor, the obtained preparations are more resistant to changes in pH (Fig. 2). After successive incubation in gastric juice and bile, they retain a high level of antylipolitic activity (Fig. 4). This index is 1.8–1.9 times higher than in the free inhibitor and 2.8–3.0 times higher than that of orlistat, which is the main active ingredient of the solely marketed medicine that is recommended to use for weight correction [14]. According to this parameter, the slightly better samples were No. 3' and No. 4'.

An important feature of food products is the value of their residual inhibitory activity closer the expiration date, which is usually 12 months. The data indicate that the samples of the biopolymer complex of oyster mushrooms help preserve a high level of antylipolitic activity even by the rational end of the period of use (Fig. 5). The best results were observed for preparations that had been obtained with a 7 % solution of sodium hydroxide both during 90 min and 270 min. (No. 3' and No. 4'). This is probably due to the extent of the antioxidant activity of their carriers, as we have found a direct correlation between these indicators [25].

The mentioned samples of the biopolymer complex facilitate higher levels of retaining antylipolitic activity of the inhibitor during immobilization (Table 2). This is determined by their chemical structure (Table 1). In particular, there is a direct correlation between the level of activity of antylipolitic preparations and their content of melanin. Since they are lipase inhibitors, their chemical structure refers them to phenolic compounds, which allows an assumption that their

similarity explains the high level of antylipolitic activity of the samples compared with the preparations obtainable by immobilizing a biopolymer-based inhibitor that is melanin-free [14].

As all the control indices showed only a slight difference between samples No. 3' and No. 4', the main selection criterion for the factors of obtaining the matrix is economic efficiency of production. Thus, it would be appropriate to obtain the matrix within a minimum processing time. Besides, the output of sample No. 4 is 7.5 % lower that of sample No. 3. The context of the task makes it rational to assume the matrix as the biopolymer complex of oyster mushrooms obtainable with a 7 % solution of sodium hydroxide within 90 minutes.

Since the increase of more than 1 % in the mass fraction of phenolic compounds in the samples has been found to have almost no effect on their inhibitory activity, it would be rational to keep this index to the specified level (Table 1). According to the research findings (Fig. 1), hydrological module 6 was selected, which corresponds to 0.17 % of the inhibitor concentration in the solution.

The findings determined the modes of certain operations for the developed technology of obtaining a dietary supplement of antylipolitic effect (Fig. 6).

For example, if the temperature of the immobilization process increases, the antypolitic activity of the preparations decreases, which may happen due to lability of phenolic compounds (Fig. 1, *c*). It also concerns the impact on factors such as duration of immobilization (Fig. 1, *b*). Therefore, high immobilization preparations should be obtained during a short exposure at room temperature.

Thus, we have determined the rational conditions of immobilizing a lipase inhibitor of vegetable origin in the biopolymer complex of oyster mushrooms: the matrix should be saturated with a 0.17 % solution of a hydrological module 6 lipase inhibitor at a temperature of +20–25 °C within 20 minutes.

The developed technology comprises several stages.

First, soluble substances are extracted from the predried and crumbled dry mushroom material through its triple treatment with boiling water of HM 10 for 60–65 minutes while stirring. Liquid extracts are separated by centrifugation. From the resulting solid residue, the acid-soluble component is extracted with the help of a 3.7 % hydrochloric acid solution of HM 5 at a temperature of +37 °C for 120 minutes while stirring. The mixture is centrifuged, and the residue is washed and centrifuged again. The resulting solid residue is processed with a 7 % alkali solution for 90 minutes while stirring. After the process, the mixture is centrifuged; then the solid residue is washed with water to the neutral pH of wash water, centrifuged again, and submitted for immobilization.

At the next stage, the rapeseed meal (the crumbled seeds after oil removal) is treated with a 95 % ethanol of HM 10 at +20–25 °C for 20–25 min. The supernatant is separated by centrifugation to remove the solvent. The resulting complex of phenolic compounds is used to prepare a 0.17 % solution.

At the last stage, the inhibitor solution is applied to saturate the biopolymer matrix. The immobilization is carried out at a temperature of +20–25 °C for 20–25 min. Then the wet mass is dried to the extent that the moisture content in the final product could be below 10 %.

Table 3 shows that the obtained dietary supplement corrects the body weight in several ways. Its antylipolitic activity reduces lipolysis in the human digestive tract; its fat-burning ability facilitates fats removal from the body; the use of the biopolymer complex dietary fibres of oyster mushrooms enhances the feeling of saturation and thus contributes to a lower intake of food. The ability to bind and excrete cholic acid from the body favourably affects lipid metabolism.

Further biomedical research can be undertaken to promote practical use of the obtained dietary supplement for body weight correction, which can confirm the supplement's effectiveness, specify its dosage, and determine the duration and manner of its use.

7. Conclusions

1. The inhibiting activity of rapeseed phenolic compounds that are immobilized in a polymer oyster mushroom matrix depends on the conditions of obtaining the latter and, accordingly, on its chemical structure. The highest levels of antylipolitic activity have been found in preparations derived from a biopolymer complex resulting from the previously prepared materials being treated with a 7 % solution of sodium hydroxide. Increased antylipolitic activity was observed when the mass fraction of the inhibitor in the samples' structure was modified from 0.5 % to 1.0 %, whereas the hydrological module was changed from 2 to 6. When temperature increased from +25 °C to +40 C and the immobilization time was extended from 20 minutes to 50 minutes, the antylipolitic activity declined.

2. Samples with an immobilized inhibitor have been found to surpass the intact form according to such properties as pH stability, thermal stability, stability under conditions simulating the gastrointestinal tract, and stability in storage.

3. The rational conditions for obtaining a biopolymer mass are the following: processing of the raw material with water, acid and a 7 % solution of sodium hydroxide for 90 minutes. The rational conditions for immobilizing consist in saturating the matrix with a 0.17 % solution of lipase inhibitor of HM 6 at a temperature of +20–25 °C for 20 minutes.

4. The developed dietary supplement contains 71.1 % of glucan, 9.6 % of chitin, 7.6 % of melanin, 3.7 % of protein, and 1 % of phenolic compounds of rapeseed. It is characterized by high levels of antylipolitic, antioxidant and enterosorption activities; moreover, it is able to stimulate growth of lactobacteria and bifidobacteria as well as to respond to the normalized indicators of microbiological safety.

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