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.
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 DSTDU 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 antilipolytic 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.

Table 1

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Conditions of obtaining</th>
<th>Content of components (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of NaOH solution (%)</td>
<td>Duration of exposure (min)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>270</td>
</tr>
</tbody>
</table>

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.
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'

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'

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.

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.

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'

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.

The properties of the dietary supplement of antilipolytic effect

<table>
<thead>
<tr>
<th>Name of the element</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass fraction of glucan, %</td>
<td>71.1</td>
</tr>
<tr>
<td>Mass fraction of chitin, %</td>
<td>9.6</td>
</tr>
<tr>
<td>Mass fraction of melanin, %</td>
<td>7.6</td>
</tr>
<tr>
<td>Mass fraction of protein, %</td>
<td>3.7</td>
</tr>
<tr>
<td>Mass fraction of phenolic compounds of rapeseed, %</td>
<td>1.0</td>
</tr>
<tr>
<td>Antilipolytic activity, ( \text{IU} / \text{g of the product} )</td>
<td>165.2</td>
</tr>
<tr>
<td>Antioxidant activity, %</td>
<td>90.3</td>
</tr>
<tr>
<td>Number of bifidobacteria, ( 10^{12} \text{ CFU/sm}^3 )</td>
<td>2.0</td>
</tr>
<tr>
<td>Number of lactobacilli, ( 10^8 \text{ CFU/sm}^3 )</td>
<td>2.8</td>
</tr>
<tr>
<td>Cholic acid sorption, mg/g of the product</td>
<td>7.7</td>
</tr>
<tr>
<td>Tannin sorption, mg/g of the product</td>
<td>7.9</td>
</tr>
<tr>
<td>Phenol sorption, mg/g of the product</td>
<td>5.7</td>
</tr>
<tr>
<td>Water-retaining ability, g/g of the product</td>
<td>6.3</td>
</tr>
<tr>
<td>Fat-binding ability, g/g of the product</td>
<td>2.1</td>
</tr>
<tr>
<td>MAFAM, ( 10^2 \text{ CFU/g} )</td>
<td>0.3</td>
</tr>
<tr>
<td>Coliform bacteria, CFU/g</td>
<td>absent</td>
</tr>
</tbody>
</table>

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.
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 antilipolytic 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 antilipolytic 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 antilipolytic 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 antilipolytic 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 antilipolitic 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 antilipolitic effect (Fig. 6).

For example, if the temperature of the immobilization process increases, the antilipolitic 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, d). 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 at 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 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 antilipolitic 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 antilipolitic 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 antilipolitic 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 antilipolitic 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 antilipolitic, 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.

References


