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Effect of pre-treatment conditions on the antiatherogenic potential of freeze-dried oyster mushrooms

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Abstract: Oyster mushroom (*Pleurotus ostreatus* L.) is a valuable food product. It possesses an antiatherogenic potential, which has to be preserved during processing. The paper features the production of oyster mushroom sublimates. It focuses on such pre-treatment conditions as grinding, disinfection, and cryostabilisation, and their effect on the antiatherogenic potential of oyster mushrooms. A set of *in vitro* experiments was performed to measure the levels of lovastatin and antioxidant, catalase, anti-inflammatory, and thrombolytic properties. Various pre-treatment conditions proved to produce different effects on the biological activity of the freeze-dried oyster mushroom product. The best results were obtained after the mushrooms were reduced to pieces of 0.5 cm, underwent UV disinfection, blanched, treated with hot air, and cryostabilised with a 1.5% apple pectin solution. The best conditions for the antioxidant properties included ozonation, UV disinfection, and cryoprotection with pectin. The critical conditions for the antioxidant properties included homogenisation, blanching, and cryostabilisation with 10% solutions of sucrose and lactose. The catalase properties did not depend on the degree of grinding and were most pronounced after ozonation. The optimal conditions for the anti-inflammatory properties included UV disinfection and cryostabilisation with lactose. Ozonation proved to be critical for anti-inflammatory properties. The optimal conditions for thrombolytic properties included ozonation and cryoprotection with a 5% sorbitol solution, while hot air disinfection proved critical. Therefore, the research provided an experimental substantiation for individual pre-treatment conditions or their combinations that turn sublimated oyster mushrooms into a valuable functional product with antiatherogenic properties.

Keywords: Oyster mushroom, freeze-drying, functional food, antiatherogenic potential, lovastatin, antioxidant properties, catalase properties, anti-inflammatory properties, thrombolytic effect

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INTRODUCTION

According to statistics, atherosclerosis and its complications remain the main cause of death worldwide [1]. The mechanisms of atherogenesis are complex and multiple. Its main causes include hyperlipidemia, oxidative stress, thrombosis, and inflammation [2].

Modern medicine is striving to find a way to curb this trend. Various therapeutic approaches are being introduced to combat atherosclerosis. However, many of them remain expensive and have various contraindications and side effects, which limits their clinical use [3]. As a result, more and more attention is given to functional food products with medicinal properties and minimal side effects. Scientists are looking for biologically active raw materials that could modify human metabolism and prevent the development and progression of atherosclerosis [4, 5].

In this regard, the oyster mushroom (*Pleurotus ostreatus* L.) is considered advantageous. Its fruit body has a high nutritional value, natural statin, and a whole complex of other biologically active substances (BAS) [6, 7]. Recent researches proved that the oyster mushroom possesses hypolipidemic, antioxidant, anti-inflammatory, and thrombolytic properties [8–11], which makes it a valuable raw material. Thus, oyster mushrooms can help to improve the existing anti-atherogenic functional foods and develop new ones.

However, the concentration and effectiveness of biologically active compounds depend not only on cultivation conditions and age of the mushrooms, but also on the processing methods [12, 13].

Some of the existing food processing technologies make it impossible to preserve the entire complex of biologically active substances [14, 15]. Today, freeze-drying is considered the least harsh and the most reliable treatment method of BAS production. It ensures stability of thermolabile and hydrolytically unstable substances, increases shelf life, and optimises storage conditions [16–18]. However, even when all the necessary regulations for freeze-drying have been observed, the properties of the product depend on the pre-processing conditions. An appropriate use of various pre-treatment methods significantly increases the efficiency of drying, improves the quality of the product, and preserves its properties [19–21]. A careless use of pre-treatment methods can lead to a decrease in the content of certain BAS in sublimates [22–24]. Thus, each raw material requires its own freeze-drying technology based on experimental data about the effect that pre-treatment conditions produce on the specific properties of the finished sublimates.

The antiatherogenic effects of freeze-dried oyster mushrooms have already become focus of scientific studies [25]. However, there have been no studies connected with the effect of pre-treatment methods on the preservation of BAS and natural antiatherogenic potential of sublimated oyster mushrooms, which adds to the relevance of the present research.

STUDY OBJECTS AND METHODS

The present research used the following chemicals: chloroform ($CHCl_3$), hydroxyamine hydrochloride ($NH_2OH{\cdot}HCl$), reduced iron, perchloric acid ($HClO_4$), ethanol (C_2H_5OH) (Sigma-Aldrich), microbiologically pure lovastatin ($C_{24}H_{36}O_5$) (TEVA, Hungary), reagent (chromogen containing an $ABTS^{\bullet+}$ radical) (Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus), trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, ammonium thiocinate (NH_4NCS), ferrous chloride ($FeCl_2$), oleic acid ($C_{18}H_{34}O_2$), hydrogen peroxide (H_2O_2), monosubstituted potassium phosphate (KH_2PO_4), disubstituted sodium phosphate (sodium hydrogen phosphate 12-water, $Na_2HPO_4{\cdot}12H_2O$) (Sigma-Aldrich), dextrose ($C_6H_{12}O_6{\cdot}H_2O$), sucrose ($C_{12}H_{22}O_{11}$) (Sigma), monohydric citric acid ($C_6H_8O_7$), sodium citrate ($C_6H_5Na_3O_5$), sodium chloride (NaCl), lactose ($C_{12}H_{22}O_{11}{\cdot}H_2O$), and sorbitol ($C_6H_{14}O_6$) (Sigma-Aldrich). All the substances were purchased from Diaem (Russia).

The study featured oyster mushroom (*Pleurotus ostreatus* L.), strain NK35 (SYLVAN, Hungary). It was harvested in 2018 and cultivated under the standard mushroom production conditions in the Stavropol Region. The fruit bodies were of the same size and maturity, undamaged. During the experiments, the fresh mushrooms were stored in a refrigerator at 5–7°C. Before the experiments, the fruit bodies were thoroughly washed under running water.

The antiatherogenic potential of the freeze-dried oyster mushroom product was evaluated *in vitro* based on the concentration of lovastatin, as well as antioxidant, catalase, anti-inflammatory, and thrombolytic properties.

The first stage featured the effect of the degree of preliminary grinding on the antiatherogenic properties of the sublimates. The grinding was conducted by reducing the fruit bodies into pieces with the side sizes of 2.0–2.5 cm and 0.5–1.0 cm. The pieces were homogenised using a laboratory Sterilmixer 12 (PBI, Italy) at No. 9 high-speed mode. Whole mushrooms served as control sample. The oyster mushroom samples were spread in one layer on separate stainless steel trays. The homogenised substance was poured into the trays to form an even layer with a thickness of ≤ 0.8–1 cm. All samples were frozen in a SE-45 refrigerator (TEFCOLD, Denmark) at –40°C for 72 h and subsequently freeze-dried.

The second stage tested the effect of preliminary disinfection methods on the preservation of antiatherogenic properties in the sublimates. The mushrooms were subjected to blanching, UV disinfection, ozonation, and hot air treatment [26, 27].

Blanching is one of the most common pre-treatment methods. It reduces microbial challenge and inactivates the enzymes that reduce the quality of the freeze-dried product. According to Galoburda *et al.*, the optimal blanching temperature regime is 70–80°C, since it provides the best drying performance for mushrooms [28]. Hence, the oyster mushrooms were blanched in water at 70 °C for 3 min, cooled under running water, and drained in a sieve for several minutes.

The UV disinfection of the oyster mushrooms was performed using an Azov portable ultraviolet irradiator, modification OBN-35-01 UHL 4.2 (Russia). The fruit bodies were put on plastic trays in one layer, placed at a distance of 60 cm from the irradiator and treated for 15 min.

The ozonation was performed using a universal ozoniser of air and water Ozone OViV (Ukraine). The ozonation was carried out in a ventilation hood at 22°C in a 10-litre chamber improvised from PVC film. The ozonation mode was based on [29] and the operation manual: ozonator power, 100%; gas flow rate, 2.0 dm^3/min; ozone concentration, 8 mg/dm^3; exposure time, 20 min.

The hot air treatment was performed using a TS-1/80 SPU dry-air thermostat (Smolensk Special Design-Technological Bureau of Software Management Systems, Russia). The mushrooms were placed on a wire shelf and kept in the thermostat under forced ventilation at 60°C for 60 min.

The third stage assessed the effect of various cryoprotectors on the atherogenic potential of the oyster mushroom sublimates. The experiment involved natural

substrates that are widely used in food industry: a 10% sucrose solution, a 10% lactose solution, a 5% sorbitol solution, and a 1.5% pectin solution. In all cases, the whole fruit bodies were soaked in aqueous solutions of the cryoprotectors (volume ratio = 1:20) for 30 min. The untreated oyster mushroom fruit bodies acted as control sample.

After the disinfections and cryostabilisations, the fruit bodies were placed on separate sheets, frozen, and freeze-dried.

All the samples were dried in an LS-500 freeze dryer (Prointech, Russia), which included a freeze dryer and a vacuum station. The glass lid of the drying chamber was covered with an opaque material to prevent degradation of antioxidants by photo-oxidation. The working pressure in the drying chamber was 80–90 Pa; the condenser temperature was 48–49°C. The temperature of the samples did not exceed 29–30°C during the entire drying process. The average drying time was 26–27 h.

The mushrooms were dehydrated until the residual moisture content was 6–8%. The moisture content in the dried oyster mushroom samples was measured using an MB 25 moisture content analyzer (Ohaus, China). The indicators were measured using the following automatic measurement mode: heating temperature = 75°C, measurement time = 5 min. The resulting oyster mushrooms sublimates were placed into a dry, hermetically sealed container and stored in dark at ≤ 25°C for further analysis.

The amount of lovastatin in the sublimates was estimated according to the authentic method using the hydroxam method after lovastatin had been extracted with chloroform and concentrated [30, 31]. The ground dried mushrooms were weighed into portions of 0.1–0.2 g, extracted with 5.0–10.0 cm^3 of chloroform, and filtered. The filtrate was evaporated using a RV 10 Basic V rotary vacuum evaporator (IKA, Germany). The remaining filtrate was diluted with 1.0 cm^3 of a 0.9 M alcohol alkaline solution of hydroxylamine and 5.0 cm^3 of a 5.73 mM solution of ferric (III) chloride. After that, pH was adjusted to 1.2 ± 0.2 with a 2M hydrochloric acid solution. The extinction of the resulting magenta solution was measured using an SF-102 spectrophotometer (Research and Development Centre NPO INTEROFOTOFIKA, Russia) at a wavelength of λ = 513 nm. The calculation was performed according to the calibration curve.

To assess the antioxidant activity of the sublimates, we measured the radical absorption and the degree of inhibition of lipid peroxidation (LPO). To assess the radical absorption, the dry oyster mushrooms were made into powder. Then the powder was extracted with bidistilled water in a shaker at 50–60°C for 3 h. The rotation speed was 190 rpm. After that, the material was filtered as described in [32]. The antioxidant activity of the extract was determined *in vitro* using the OxiStat test system (Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus). It was a one-stage assessment of reduction value of the resulting $ABTS^{\bullet+}$ radical by antioxidants. The scheme is described in [33] as follows: $ABTS^{\bullet+} + AO \rightarrow ABTS + AO^{\bullet+}$.

When antioxidants interacted with $ABTS^{\bullet+}$, the optical density of the solution of the cation radical fell down to 600–800 nm in proportion to the concentration and activity of the antioxidant. The optical density was measured using a spectrophotometer at a wavelength of 675 nm. The optical path length of the cuvettes was 1.0 cm.

To provide a quantitative assessment of the antioxidant activity, we used trolox, i.e. a standard antioxidant, which is a water-soluble analogue of vitamin E:

$$\% \text{ inhibition} = 100(1-\Delta A_o/\Delta A_c) \quad (1)$$

$$AA = [C_{st}]/\% \text{ standard inhibition} \times \times \% \text{ sample inhibition} \quad (2)$$

where:

AA – antioxidant activity;

ΔA_o – optical density of the experimental sample;

ΔA_c – optical density of the control sample (buffer);

C_{st} – standard concentration (trolox).

The radical absorption results were expressed in mg of trolox equivalent per gram of dry matter (mg TE/g)

To evaluate the *LPO* inhibition activity, 0.1 g of powdered dry mushrooms was added to 2.0 cm^3 of bidistilled water. After 24 h of maceration at room temperature, the extract was filtered and centrifuged at 1300 rpm for 10 min. The LPO inhibition activity of the obtained extract was measured in an oleic acid emulsion system according to the slightly modified procedure described in [34]. 0.1 cm^3 of the extract was added to 4.0 cm^3 of phosphate buffer (50 mM, pH 7.0), and 0.1 cm^3 of oleic acid was added to 4.0 cm^3 of ethanol (95 wt%, aqueous solution). The total volume was brought to 10.0 cm^3 with distilled water, mixed in a sealed conical tube with a screw cap, and incubated at 40°C in the dark for 7 days. The oxidation state was evaluated using iron thiocyanate at 24 h intervals. The reaction solution (100 μL) was mixed with 4.7 cm^3 of ethanol (75 wt%, aqueous solution), 0.1 cm^3 of an ammonium thiocyanate aqueous solution (30% w/v), and 0.1 cm^3 of an iron chloride (II) solution (20 mM in 3.5% (v/v) HCl). After 3 min, the absorbance was measured at a wavelength of 500 nm using a UV spectrophotometer. An increase in optical density meant an increase in the level of oleic acid oxidation. Trolox (0.95 mmol/dm^3) was used as a reference. The blank sample contained deionised water instead of the extract.

When calculating both the antiradical activity and the activity of LPO inhibition, we took into account the fact that the extracts had their own colour, which absorbed a particular wavelength in the visible spectrum.

The catalase activity of oyster mushroom sublimates was measured using a modified technique based on the Beers and Sizer spectrophotometric method [35].

This fast and accurate analysis presupposes a hydrogen peroxide dehydrogenation and determination of its loss at λ = 240 nm. The powdered oyster mushrooms were weighed into portions of 0.020–0.025 g and extracted with 5.0 cm^3 of chilled 50 mM phosphate buffer with pH = 7.0 for 15 min in the cold with periodic stirring. The extract was filtered and centrifuged at 4°C for 20 min and acceleration of 1200 g. The experimental solution contained 1.0 cm^3 of 50 mM phosphate buffer with pH=7.0 and 1.0 cm^3 of 0.1% hydrogen peroxide. Its optical density (D_1) was measured at 240 nm relative to the control solution, which contained 2.0 cm^3 of 50 mM phosphate buffer. After filtration, 0.1 cm^3 of the sublimate sample extract was introduced into the experimental sample. In addition, 0.1 cm^3 of extract was added to the control solution. The optical density of the experimental solution was determined after 30 s (D_2).

The enzymatic activity was calculated for 1 mmol of substrate (H_2O_2) split in 1 min with 1 g of sublimate sample according to the following formula:

$$A= ((D_1-D_2)V_1 \cdot n)/D_1 \cdot m \cdot V_2 \cdot t) \quad (3)$$

where:

A – enzymatic activity, mmol/g·min;

D_1 – optical density of the hydrogen peroxide solution before the extract was introduced;

D_2 – optical density of the hydrogen peroxide solution after incubation with the extract;

V_1 – total extract volume, cm^3;

n – amount of hydrogen peroxide introduced, mmol;

m – weight of oyster mushroom sublimate in the extract, g;

V_2 – volume of extract for analysis, cm^3;

t – incubation time, min.

The anti-inflammatory activity of the oyster mushroom sublimates was determined *in vitro*. It employed the method used for assessing the osmotic resistance of erythrocyte membranes [36]. The dry mushroom sublimate was made into powder, suspended in distilled water at a concentration of 5.0 mg/cm^3, and incubated at 4°C for 12 h. The suspension was centrifuged at 7000 rpm for 10 min, after which the supernatant was filtered. Blood was obtained from healthy white laboratory Wistar rats and mixed in a 1:1 ratio with Alsever solution. The latter contained equal volumes of aqueous solutions of 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride. The resulting solution was centrifuged at 4000 rpm for 10 min. The precipitated cells were washed with physiological saline and centrifuged three time until the red blood cells were 10% by suspension volume in physiological saline. The extracts of oyster mushroom sublimates were separately mixed with 1.0 cm^3 of phosphate buffer, 2.0 cm^3 of hypotonic sodium chloride solution (0.42%), and 0.5 cm^3 of red blood cell suspension. The control sample contained 2.0 cm^3 of distilled water instead of the hypotonic solution. The mixes were incubated at 37°C for 20 min and centrifuged at 3000 rpm.

After that, the supernatant liquid was decanted, and the haemoglobin content was estimated using a spectrophotometer at λ = 560 nm. The percentage of resistance of red cell membranes was assessed based on the fact that the haemolysis obtained in the control sample was 100%. It was calculated by the formula:

Percentage of resistance = 100 – (optical density of the experimental sample/optical density of the control sample) × 100

To assess the thrombolytic activity of the sublimates, blood obtained from white Wistar rats was distributed into different pre-weighed sterile microcentrifuge tubes (0.5 cm^3 in each) and incubated at 37°C for 45 min. After the clot was formed, the serum was completely removed without disturbing the clot, and each tube was again weighed to calculate the weight of the clot. 100 μL of sublimate extract was added into each tube with a pre-weighed clot. All tubes were incubated at 37°C for 90 min. After incubation, the released liquid was removed, and the tubes were weighed again. The difference in weight before and after clot dissolution was expressed as a percentage [37].

The content of substances and their activity were expressed in terms of absolute dry raw materials. All quantitative parameters were triplicated. The results were recorded as arithmetic mean ± standard error of the arithmetic mean (M ± m) and subjected to statistical processing using the method of one-way ANOVA test and the Biostat software (version 4.03). The significance of the differences was measured at $P \leq 0.05$.

RESULTS AND DISCUSSION

A single-phase ANOVA was conducted to compare the quantitative values of the properties responsible for the antiatherogenic potential of the freeze-dried oyster mushroom product. It also made it possible to check whether there was any significant difference in these properties after various pre-treatment methods.

As a potential antiatherogenic product, the oyster mushrooms were checked for the concentration of lovastatin. This natural statin reduces the production of endogenous cholesterol as it inhibits the activity of hydroxyl-3-methyluracil-coenzyme reductase [38]. The experiment took into account the level of antioxidant and catalase activities that resist the accumulation of excess reactive oxygen. Together with excessive lipids in the blood, reactive oxygen is known to cause atherosclerosis [39]. We tested the abilities of freeze-dried oyster mushrooms to inhibit the inflammation and thrombosis. They are considered the key pathogenetic mechanisms of atherosclerosis as they facilitate the transformation of risk factors into morphological changes [40].

A set of experiments was performed to define the effect of disinfection, cryoprotection, and various degrees of preliminary grinding on the safety and activity of the abovementioned properties.

Table 1 Effect of preliminary grinding of oyster mushroom fruit bodies on the bioactive properties of freeze-dried product (M ± m)

№	Grinding size	Lovastatin, mg/kg	Radical absorption activity, mgTE/g	Catalase activity, mmol/g·min	Anti-inflammatory effect, %	Thrombolytic activity, %
1	Whole fruit bodies (control sample)	316.2 ± 8.3[a]	9.6 ± 0.3[a]	17.9 ± 0.6[a]	34.6 ± 0.8[a]	15.2 ± 0.4[a]
3	Pieces with side size 2.0–2.5 cm	305.6 ± 7.6[a]	9.1 ± 0.3[a]	18.7 ± 0.6[a]	35.1 ± 0.7[a]	14.1 ± 0.3[a]
4	Pieces with side size 0.5–1.0 cm	298.4 ± 6.1[a]	9.4 ± 0.4[a]	17.3 ± 0.5[a]	36.4 ± 0.9[a]	12.4 ± 0.3[b]
5	Homogeneous state	174.8 ± 4.2[b]	5.2 ± 0.2[b]	16.9 ± 0.5[a]	34.1 ± 0.8 [a]	9.9 ± 0.2[c]
6	Sources	50.0–505.0 (*Pleurotus ostreatus*) Gunde-Cimerman *et al.* [43]; 165,3–606,5 Chen *et al.* [44]	0.61–14.07 (*Pleurotus citrinopileatu*) Nattoh *et al.* [45]	14.66 (*Pleurotus Ostreatus*) Susmitha *et al.* [46]	18.66–43.50 (*Pleurotus florida*) Pandimeena *et al.* [47]; 54,33–85,12 (*Pleurotus florida*) Varghese *et al.* [36]	18.62 (*Pleurotus ostreatus*) Islam *et al.* [8]

Mean values with different letters in the same column are statistically different (P < 0.05). mgTE/g

Thickness, shape, and volume ratio of samples are known to affect the drying rate and quality of the finished product [41].

The whole dried oyster mushroom fruit bodies selected as control sample were tested for the abovementioned properties. The obtained quantitative indicators appeared to be comparable with the data for *Pleurotus* mushrooms presented in previous studies (Table 1). When the fruit bodies were ground to pieces with side sizes of 2.0–2.5 cm and 0.5–1.0 cm at the pre-treatment stage, it did not affect the concentration of lovastatin in the freeze-dried product. However, the content of lovastatin in the homogenised sublimates turned out to be 45% less than in the whole-dried samples. The technological process of homogenisation probably reduced the degree of heterogeneity of the distribution of chemicals and phases by volume. It might have changed the sensitivity of lovastatin to the conditions of the subsequent stages of lyophilisation. In addition, homogenisation is known to cause a shift in the pH of raw materials. According to Piecha, when pH of the medium increases, the lactone structures of statins can be partially or completely converted to the corresponding forms of hydroxyacids [42].

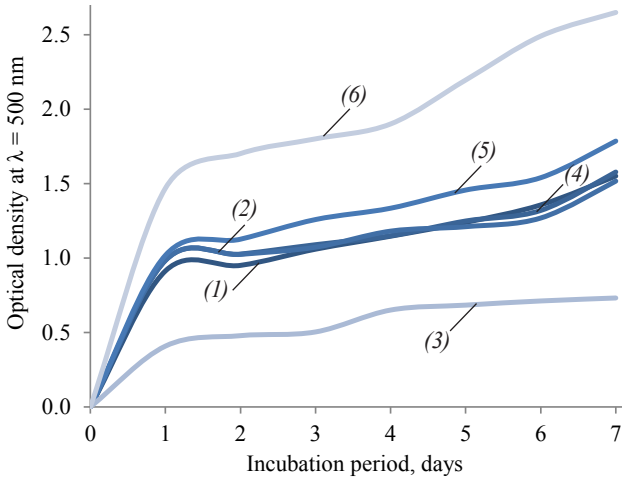
The assessment of the effect of grinding size on the preservation of antioxidant properties had a similar result. Homogenization reduced the activity of radical absorption by almost 46%. The homogenised samples also demonstrated minimal LPO inhibition activity (Fig. 1).

The data were consistent with some studies that featured the effect of homogenisation during freeze-drying of berries [19]. According to Paciulli *et al.*, the results may be explained by the fact that large tissue damage caused a loss of antioxidant substances [48].

However, the preliminary grinding of oyster mushroom affected neither catalase nor anti-inflammatory properties of its sublimates.

Pleurotus mushrooms owe their thrombolytic properties to the high level of biosynthesis of the protease enzyme complex. Proteases have an affinity for fibrin and cause its lysis [49]. The thrombolytic properties of freeze-dried oyster mushrooms depended on the degree of grinding at the pre-treatment stage: the experiment showed a statistically significant decrease as the fruit bodies were ground into smaller pieces. The samples subjected to preliminary homogenisation demonstrated the lowest thrombolytic activity. Such result might be connected with the fact that cellular disruption facilitates interaction between proteolytic enzymes and extracellular protease inhibitors.

Although pre-treatment grinding may facilitate the drying process, it proved irrational in terms of preservation of lovastatin and other antioxidant and thrombolytic substances [41].



(1) No treatment
(2) 0.5–1.0 cm pieces
(3) Trolox
(4) 2.0–2.5 cm pieces
(5) Homogeneous sample
(6) Blank sample

Figure 1 Effect of preliminary grinding on the LPO inhibition of the freeze-dried product. (Note: in Figs. 1–3, a lower optical density at 500 nm corresponds to a higher LPO inhibition)

Table 2 Effect of various methods of preliminary disinfection on the bioactive properties of freeze-dried product (M ± m)

№	Disinfection	Lovastatin, %	Radical absorption activity, mgTE/g	Catalase activity, mmol/g×min	Anti-inflammatory action, %	Thrombolytic activity, %
1	No treatment (control sample)	316.2 ± 7.9[a]	8.8 ± 0.3[a]	16.0 ± 0.4[a]	35.2 ± 0.8[a]	15.7 ± 0.4[a]
2	UV	308.4 ± 8.1[a]	10.3 ± 0.4[b]	15.6 ± 0.4[a]	65.1 ± 1.6[b]	14.8 ± 0.4[a]
3	Ozonation	217.4 ± 7.1[b]	9.4 ± 0.3[b]	21.6 ± 0.5[c]	6.3 ± 0.5[c]	23.9 ± 0.6[b]
4	Blanching	311.3 ± 8.1[a]	4.6 ± 0.2[c]	17.1 ± 0.5[a]	33.2 ± 0.8[a]	16.1 ± 0.5[a]
5	Hot air	299.4 ± 6.5[a]	8.4 ± 0.3[a]	1.6 ± 0.1[b]	36.4 ± 1.0[a]	10.6 ± 0.3[c]

Mean values with different letters in the same column are statistically different ($P < 0.05$)

Food security is as important as its nutritional and biological value. Microbiological contamination is an indicator of food security. Therefore, disinfection is a necessary pre-treatment stage. Blanching and hot air treatment had no statistically significant effect on the content of lovastatin in the finished sublimates if compared with the control samples (Table 2). UV disinfection may cause photodegradation of statins. However, it also demonstrated no significant changes in the concentration of lovastatin in the sublimates. The only difference was a slight decrease in the content of lovastain, which is consistent with the results obtained by [42], according to which lovastatin proved to be the most UV resistant statin.

The ozonation resulted in a significant loss of lovastatin. Its concentration in the sublimates decreased by 31.4% compared to the control samples, which confirmed the data published in [50], according to which oxygen makes lovastatin instable.

UV disinfection and ozonation resulted in a higher radical absorption and LPO inhibition (Fig. 2). Such results are consistent with other studies [51–53] that proved a better preservation, and sometimes even an increase, of antioxidant substances in mushrooms and fruits after ozonation and UV disinfection. According to Sudheer *et al.*, ozone can trigger the formation of such secondary metabolites as phenols and flavonoids [54].

Hot air disinfection caused no statistically significant changes in the antiradical activity and LPO inhibition (Fig. 2). These results contradicted with those described in [55]. On the one hand, the effect might be explained by the thermally induced extraction of previously bound or polymerized molecules of antioxidants, in particular, phenols. On the other hand, it may be due to the inactivation of enzymes involved in their catabolism, as demonstrated by recent studies of vegetable drying processes [56]. In addition, the obtained results might be explained by the fact that hot air treatment can trigger the formation of new compounds, e.g. Maillard reaction products, which possess good antioxidant properties [57].

The blanching produced a significant decrease in the antiradical activity of the sublimates. Its value was 47.7% lower than that of the control sample. The results confirmed the data described in [58, 59]. According to Lam *et al.* and Radzki *et al.*, leaching and a low ability to absorb oxygen radicals resulted in a lower concentration of antioxidant substances after preliminary blanching. In addition, the blanched sublimates showed a minimal LPO inhibition [58, 59].

Various disinfection methods produced different results on the level of catalase activity of the sublimates. Hot-air treatment resulted in the lowest catalase activity. These results confirmed those described in [60], according to which a higher drying temperature reduced the residual activity of the oyster mushroom catalase enzyme.

The ozonation produced the highest catalase activity. These results confirmed those described in [61, 62], which showed an increase in the catalase activity of fruits after ozonation. The increase was explained by the fact that ozone came into contact with the biological tissue of mushrooms and caused oxidative stress, which was accompanied by activation of various antioxidant enzyme systems, e.g. catalase.

Contrary to previous assumptions [63], the blanching caused no changes in the level of catalase activity. According to Egbebi *et al.*, blanching of mushrooms inactivated catalase. In our opinion, it can be explained by the relatively short blanching time [63]. This presumption corresponds with the observations published in [64], which described catalase inactivation only in cases when blanching lasted > 10 min.

Various disinfection methods produced various effects on the anti-inflammatory activity of the product

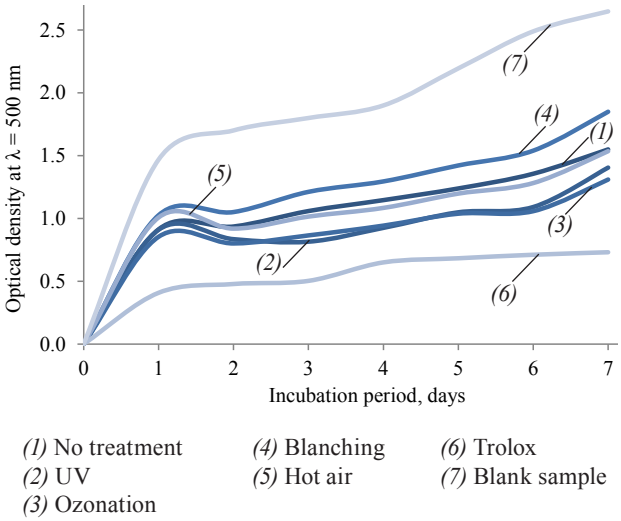


Figure 2 Effect of various methods of preliminary disinfection on the LPO inhibition of the freeze-dried product

that had undergone a spray-freeze drying. The anti-inflammatory properties were determined using the erythrocyte membrane stabilisation test. The UV-treated samples showed the highest anti-inflammatory activity. Mushrooms owe most of their anti-inflammatory properties to polysaccharides, especially glucans [65, 66]. Thus, UV treatment served as an elicitor that increases production of extracellular polysaccharides in mushrooms. In addition, UV treatment might have produced phenolic compounds that produce a protective effect on biological membranes [67].

The ozonation resulted in the lowest anti-inflammatory activity. This effect might have been caused by ozone-induced oxidative reactions. According to Mzoughi *et al.*, ozone-induced oxidative reactions lead to the selective depolymerisation of polysaccharides, followed by a possible increase or, conversely, a decrease in their biological activity [68].

The thrombolytic properties also proved to depend on the methods of preliminary disinfection. Thus, the maximum thrombolytic properties were manifested in the ozonised samples. This result might have been caused by the ability of ozone to inactivate protease inhibitors [69]. The minimal thrombolytic properties were detected in the samples that had been treated with hot air. According to Ali *et al.*, protease inhibitors in mushrooms are thermally stable [70]. According to Rai *et al.* and de Castro *et al.*, proteases demonstrate the maximum activity at 55–60°C and may be wasted on the autohydrolysis of proteins heating [71, 72].

Freezing is an obligatory stage of freeze-drying. Freezing can damage the cell structure with ice crystals. The degree of damage depends on the size of the crystals and the heat transfer rate. It can affect the rheological and textural properties of the product, as well as redox processes in favour of oxidation. As a result, the number and the biological activity of the substances in the product may change. Therefore, a stabilising cryoprotector should be applied before freezing. Cryoprotectors maintain the quality of the frozen product by increasing the reversibility of the process and preserving BAS [73]. In this regard, we assessed various natural cryoprotectants and their effect on the anti-atherogenic properties of the freeze-dried oyster mushrooms (Table 3).

The chemical analysis demonstrated that the cryoprotectors had a different effect on the amount of lovastatin in the mushroom sublimates. The samples pretreated with a 1.5% solution of pectin showed no statistical changes in the concentration of oxidation-sensitive natural statin. However, there was a clear tendency to its increase. It confirmed the results published in [74], which proved that insoluble polysaccharides effectively inhibit oxidation processes in frozen semi-finished products.

The samples pretreated with a 5% sorbitol solution demonstrated a 47.6% decrease in lovastatin. The samples pretreated with a 10% sucrose solution and a 10% lactose solution appeared to contain no lovastatine. It was probably due to the hydrolysis of lovastatine in the aqueous medium of the cryoprotectors. Thus, freezing did not prove to be a limiting factor with respect to lovastatin concentration. Hence, cryoprotectors are not obligatory in this aspect.

1.5% pectin solution proved to be the best cryoprotector for oyster mushrooms as it ensured the maximum preservation of antiradical and LPO inhibition (Fig. 3). The results were consistent with [75], according to which pectin has a greater water absorption capacity compared with sorbitol and monosaccharides. The results may be attributed to the antioxidant properties of pectin itself, since its diffused part could enhance the antioxidant properties of the obtained dry product. According to Kopjar *et al.*, if added to bioactive substances containing phenolic compounds, pectin provides a synergistic effect on their antioxidant properties [76].

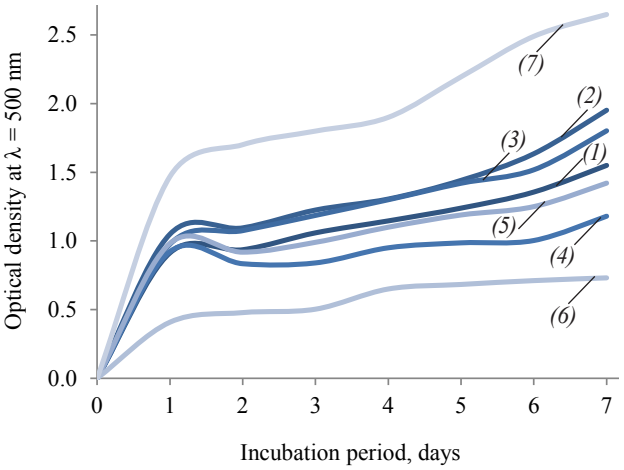
10% solutions of lactose and sucrose resulted in a significant decrease in the level of antiradical activity and LPO inhibition. On the one hand, these results may be explained by the extraction of antioxidant substances into the aqueous solutions of the cryoprotectors. On the other hand, the decrease might have been caused by the cryoprotective effect itself, since it reduces both ice crystal formation in the mushroom tissue matrix and damage to the cell structure. According to Yang *et al.*, cryoprotective effect increases extraction of antioxidant substances, e.g. phenolic compounds, from cells [77].

Cooling is known to reduce the catalase properties of certain substances [78]. However, catalase activity decreased significantly in all the experimental samples. Its value was minimal in the sublimates pretreated with a 10% lactose solution and a 5% sorbitol solution.

Table 3 Effect of pre-treatment with cryoprotectors on the bioactive properties of freeze-dried product (M ± m)

№	Cryoprotector	Lovastatin, mg/kg	Radical absorption activity, mgTE/g	Catalase activity, mmol/g·min	Anti-inflammatory action, %	Thrombolytic activity, %
1	No treatment (control sample)	310.3 ± 7.6^{a}	7.9 ± 0.3^{a}	17.1 ± 0.5^{a}	30.3 ± 0.9^{a}	14.9 ± 0.4^{a}
2	10% sucrose solution	–	5.4 ± 0.2^{b}	9.5 ± 0.4^{b}	29.4 ± 0.8^{a}	15.4 ± 0.4^{a}
3	10% lactose solution	–	5.6 ± 0.2^{b}	2.9 ± 0.1^{c}	56.4 ± 1.5^{b}	16.0 ± 0.5^{a}
4	1.5% pectin solution	339.2 ± 8.5^{a}	15.5 ± 0.5^{c}	7.0 ± 0.3^{d}	31.8 ± 1.0^{a}	14.6 ± 0.3^{a}
5	5% sorbitol solution	152.4 ± 5.9^{b}	7.5 ± 0.3^{a}	3.1 ± 0.1^{c}	28.6 ± 1.1^{a}	25.1 ± 0.6^{b}

Mean values with different letters in the same column are statistically different ($P < 0.05$)



(1) No treatment
(2) 10% sucrose solution
(3) 10% lactose solution
(4) 1.5% pectin solution
(5) 5% sorbitol solution
(6) Trolox
(7) Blank sample

Figure 3 Effect of pre-treatment with cryoprotectors on the LPO inhibition of the freeze-dried product

The maximum anti-inflammatory activity was manifested in the sublimates pretreated with a 10% lactose solution. The results confirmed those published in [79], according to which lactose proved to be a more advantageous cryoprotector than sorbitol or sucrose when used in freeze-drying of liposome preparations. It can be explained by the fact that disaccharides produce a greater stabilising effect on cell membranes during freezing than other cryoprotectors, thereby preserving polysaccharides and glycoproteins of cell membranes.

As for the thrombolytic properties, sublimates pretreated with sorbitol showed the best results. Unlike mono-, di-, and oligosaccharides, sorbitol can penetrate into cells [80]. It protects intracellular proteases and their fibrinolytic properties from possible denaturation caused by low temperature.

CONCLUSION

A set of biochemical experiments was performed to study the effect of various pre-treatment conditions on the biologically active properties that provide the antiatherogenic potential of freeze-dried oyster mushrooms. The antiatherogenic properties under study included the content of natural statin (lovastatin), as well as antioxidant, catalase, anti-inflammatory, and thrombolytic properties. The results showed that each pre-treatment method produced a different effect on the abovementioned properties of the freeze-dried product.

The experiments demonstrated that the best results for lovastatin were obtained when the raw material was ground to pieces with a side size of ≥ 0.5 cm, subjected to UV disinfection, blanched, treated with hot air, and cryoprotected with a 1.5% pectin solution.

As for the antioxidant properties, such as radical absorption and LPO inhibition, the best conditions included UV disinfection, ozonation, and cryoprotection with a 1.5% pectin solution. Homogenisation, blanching, and cryostabilisation with 10% solutions of sucrose and lactose were found critical for antioxidant properties.

The catalase activity of the product did not depend on the degree of grinding, blanching, and UV disinfection. It was maximal after ozonation. The list of critical pre-treatment conditions included hot air treatment and exposure to all the cryoprotectors except pectin.

The anti-inflammatory properties were best preserved after UV disinfection and cryoprotection with a 10% lactose solution. Ozonation appeared to be the only critical pre-treatment factor.

The best results for thrombolytic properties were obtained when the oyster mushrooms were ozonated and cryoprotected using a 5% sorbitol solution. Critical factors included homogenisation and hot air treatment.

Thus, the experiments revealed advantages of individual pre-treatment conditions and their combinations. The applied conditions can turn freeze-dried oyster mushrooms into a functional food product or ingredient. The new functional product significantly improved the properties that affect such pathogenetic factors of atherogenesis as hyperlipidemia, oxidative stress, inflammatory reaction, and thrombosis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests related to this article.

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