# Intensification of the process of ergosterol accumulation by yeast *Saccharomyces cerevisiae* by changing the composition of the culture medium and using ultrasound

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**Abstract.** The chemical synthesis of vitamin D2, an important nutrient for living organisms, is complex due to the asymmetric structure of the vitamin molecule. This opens up significant prospects for the development of biotechnological approaches to cyanocobalamin synthesis, which forms the basis of this work. The study considers the possibility of using nutrient media of different compositions, as well as ultrasound exposure to intensify the biosynthesis of ergosterol by yeast *Saccharomyces cerevisiae* as a precursor of vitamin D2. The standard YEPD medium is considered as a nutrient medium, as well as low-carb and sodium nitrate dilution. The modes of ultrasonic exposure varied in power and processing time, 9 variants were evaluated. Together, the intensification of the process of ergosterol biosynthesis by yeast cells can ensure the economic viability, stability, and expediency of its production on an industrial scale. The research results have shown the expediency of the proposed approaches. Thus, the introduction of sodium nitrate into the nutrient medium allowed for an increase in synthesized ergosterol in the yeast biomass by 18.2% compared with the standard medium. Optimization of ultrasonic treatment modes has shown the possibility of achieving the synthesis of ergosterol to a content of 11.82% per dry matter.

## 1 Introduction

An indisputable fact today is the need of the human body for minor biologically active substances, the sources of which can be food products and biologically active additives. Among such substances: vitamins and their precursors, minerals, phenolic compounds and others. Minor biologically active substances can have a pronounced beneficial effect on human health through participation in various metabolic processes.

Thus, the precursor of vitamin D2, ergosterol, increases the body's ability to resist diseases, has antibacterial and antitumor effects. It is widely used for the prevention of rickets, osteoporosis, tetania, animal diseases, etc. As a feed additive, it can significantly

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increase the egg production and incubation period of poultry. It is also an intermediate for some new drugs that prevent the occurrence of cardiovascular diseases, suppress tumors, eliminate lung diseases, as well as diseases associated with impaired blood pressure and weakening of the human immune system. Ergosterol is a sought-after ingredient and has a wide demand both in the Russian market and abroad.

The ergosterol conversion product, vitamin D2, can also be used as a feed additive to increase egg production and incubation potential of livestock and poultry. In addition, ergosterol can be used as a pharmaceutical chemical raw material for the production of steroid drugs such as cortisone and the hormone progesterone [1-2].

Through in-depth research and development, the potential value of ergosterol can not only meet the growing demand, but also contribute to the development of medicine and bioindustry, providing more opportunities for the introduction of effective technologies for the future of healthcare and manufacturing.

Being a safe, nutritious, and healthy natural product, ergosterol increasingly meets people's expectations regarding the consumption of high-quality ingredients that do not pollute the environment.

In the pharmaceutical industry, ergosterol is used both independently and to produce vitamin D2. Maintaining the supply of provitamin ergosterol and vitamin D2 to the population is a global public health priority in many countries of the world due to the prevention of calcium-phosphorus and bone metabolism disorders, as well as the early development and severe course of many socially significant diseases such as cardiovascular, autoimmune, respiratory, malignant neoplasms, obesity and infections [3-5].

This compound not only has unique physiological effects, but is also widely used in drug development. As an important component of fungal cell membranes, ergosterol plays an important role in cell viability, membrane fluidity, activity of membrane-bound enzymes, membrane integrity and transport of cellular material [1, 5-7].

Ergosterol is thus widely used in medicine, chemical and food industries. It is an important metabolite with potentially high economic and research value. The study of ergosterol and ways to intensify its synthesis are of great importance.

Currently, ergosterol is mainly synthesized by microbial fermentation, but in recent years new scientific developments have emerged to isolate this compound from some plants. In recent years, some scientists have also extracted ergosterol from mycelial fungi.

Ergosterol is an important component of *Saccharomyces cerevisiae* yeast cell membranes and plays an important role in ensuring yeast viability, membrane permeability and fluidity, membrane-bound enzyme activity, membrane integrity and material transport through the cell wall. Ergosterol can interact with fatty acids to form ergosterol esters and store them in lipid droplets to maintain the balance of sterols in cells and regulate the efficiency of phospholipase transport [8]. Ergosterol can also affect the absorption and use of nutrients by regulating the activity of membrane-bound ATP, is able to stimulate the growth and proliferation of yeast cells and is considered a "yeast hormone". At the same time, the stress reaction of ergosterol plays an important role in the fermentation process. A sufficiently high tolerance of yeast to changing external conditions is closely related to the ergosterol level in yeast cell. Ergosterol levels are very important for hypoxic reaction and temperature stress in *Saccharomyces cerevisiae* [9-11]. Under hypoxic conditions, the growth and metabolism of *Saccharomyces cerevisiae* are suppressed, yeast can activate its ergosterol biosynthesis pathway to increase alcohol biosynthesis and promote its accumulation.

The pathway of ergosterol synthesis in a yeast cell is a rather complex process involving the participation of many enzymes and consuming a lot of energy. The synthesis of one ergosterol molecule consumes at least 24 ATP molecules and 16 HADPH molecules. The pathway of ergosterol biosynthesis in yeast can be divided into three stages: mevalonate biosynthesis, farnesol pyrophosphate biosynthesis, and ergosterol biosynthesis [3, 7-10].

Considering the growing demand for ergosterol every year, industrial production processes need the introduction of intensification technologies and the use of modern approaches. Thus, the development and use of modern technologies for the production and intensification of the ergosterol production process is of great economic importance [3-5, 9-12].

In recent years, interest in the yeast cell as a source of ergosterol has increased significantly. An active search is underway for approaches to intensify both the process of ergosterol biosynthesis and the search for methods for effective extraction of ergosterol from a yeast cell.

In this part, ultrasound becomes an attractive modern approach. The technology of ultrasonic exposure in biotechnological processes is becoming more widespread due to its efficiency, environmental friendliness, and safety. The ultrasound effect on ergosterol synthesis in yeast includes complex biochemical and biophysical processes. Ultrasound, as a mechanical wave, can cause acoustic and sonochemical effects in liquid media, thereby affecting biosynthetic reactions. The following are some possible mechanisms of ultrasound action on ergosterol biosynthesis [13]:

(1) Increased permeability of cell membranes: ultrasound can cause rupture of the cell membrane or increase its permeability, which facilitates the penetration of substrates (such as, for example, ergosterol precursors) to enter the cell, thereby contributing to the process of yeast synthesis of ergosterol.

(2) Mixing the substrate and increasing the reaction rate: ultrasound can promote the mixing of substrates and enzymes, increase the reaction rate and thus accelerate the process of ergosterol yeast synthesis.

(3) Stimulation of cellular metabolism: ultrasound can stimulate metabolic activity in cells, including enhancing the activity of the biosynthetic pathway necessary for ergosterol yeast synthesis, thereby increasing the ergosterol production.

(4) Destruction of enzymes or cellular structure: high-intensity ultrasound can destroy active enzymes or the cellular structure of cells, affecting the process of ergosterol yeast synthesis.

(5) Temperature rise: Ultrasonic waves can cause a local temperature rise, which can affect the reaction rate and the yield of ergosterol yeast synthesis.

Thus, the study of the effect of the digestive medium composition and the effect of ultrasound in various modes on the ergosterol yeast biosynthesis with subsequent optimization of this process is an urgent task, potentially allowing to significantly increase the yield of the target product, which is the purpose of this study.

## 2 Materials and Methods

### 2.1 Metrics of the cultivation process

Materials of the experiment:

Experimental yeast *Saccharomyces cerevisiae* commercially available were purchased in a retail chain in the city of Chelyabinsk.

Nutrient medium:

3 types of nutrient medium were used for yeast cultivation:

- standard YEPD nutrient medium: yeast extract, peptone, and dextrose (sterilization 30 min).
- standard YP nutrient medium: yeast extract and peptone, without adding a carbohydrate component (sterilization 30 min);

- standard YEPD nutrient medium with the addition of sodium nitrate as a stimulant of the ergosterol biosynthesis process (sterilization 30 min).

All samples were cultured under strictly aerobic conditions for 12 hours.

Experiment methods:

The determination of the sterol content was carried out by extracting 70% ethyl alcohol from the yeast substrate. The calculation of the quantitative content of the desired substance was determined by the optical density in concentrated sulfuric acid at an analytical wavelength of the spectrophotometer 328 nm. The conversion was performed to ergosterol. The optical density was measured immediately after the solution preparation. The calculation of the sterol content in terms of ergosterol was carried out on absolutely dry raw materials as a percentage.

To assess the ultrasound effect on the process of ergosterol biosynthesis, a two-factor regression analysis was used with the processing of the results in the MathCad 14.0 program. In the experiment, the effect of ultrasound power and exposure time on the amount of ergosterol was monitored. The following combination of factors was used (Fig. 1).

$$X1 := \begin{pmatrix} 189 \\ 315 \\ 441 \\ 189 \\ 315 \\ 441 \\ 189 \\ 315 \\ 441 \end{pmatrix} \quad X2 := \begin{pmatrix} 1 \\ 3 \\ 5 \\ 3 \\ 5 \\ 1 \\ 5 \\ 1 \\ 3 \end{pmatrix}$$

**Fig. 1.** The combination of ultrasonic exposure factors used (X1 – ultrasonic power, W; X2 – exposure time, min).

## 3 Results and Discussion

Currently, using labeled acetate, it has been unequivocally proven that the initial building block in the synthesis of sterols is acetyl-CoA. Currently, the conditions under which sterol formation occurs in yeast organisms are relatively well studied. Aeration is the main factor that dramatically changes the level of sterol formation in yeast cells of the genus *Saccharomyces* [1, 5, 15]. In the presence of oxygen, the synthesis of sterols is carried out very quickly. At the same time, the composition of the nutrient medium used for cultivation is also crucial for the final yield of ergosterol. Sodium nitrate was used as a source of minerals.

The obtained results are shown in Fig. 2.

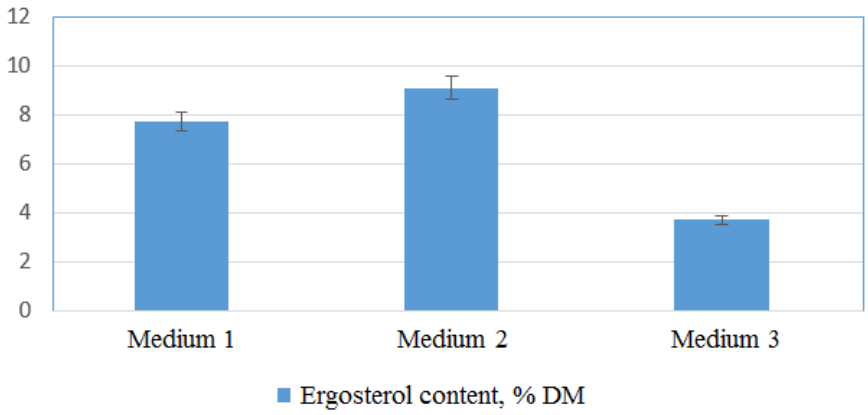
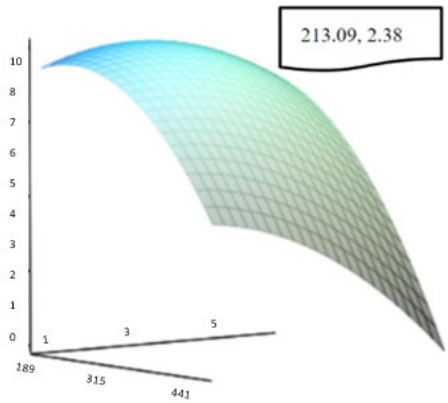



**Fig. 2.** Ergosterol content in yeast biomass depending on the type of nutrient medium used.

From the results of the experiment, it can be concluded that the experimental sample 2, containing sodium nitrate, has the highest content of ergosterol extracted from yeast cells, which is 9.1%. The difference between the sample cultivated in medium 3 was the greatest, which may be due to the ratio of nutrients in the culture medium, namely the absence of a carbohydrate component. It is known that an excessive amount of nitrogen in the nutrient medium in comparison with the carbohydrate component slows down the ergosterol synthesis.

At the next stage of the research, the ultrasound effect on the process of ergosterol biosynthesis by yeast was evaluated. The content of ergosterol synthesized by *Saccharomyces cerevisiae* was measured at various ultrasound powers and times, and the experimental data were processed using two-factor regression analysis. After conducting a number of studies and statistical processing of the results, we obtained a response surface and a regression equation that fully describe the effect of exposure to ultrasound treatment modes of different power and time on ergosterol biosynthesis in yeast. The response surface is shown in Figure 3.




$$Y = 1.523 \cdot 10^{-5} X_1^2 + 0.606 X_2^2 + 6.782 \cdot 10^{-4} \times X_1 X_2 + 0.009 X_1 + 1.266 X_2 + 2.418$$

**Fig. 3.** Results of optimization of the conditions for processing yeast biomass with ultrasound (opt: power, X1 = 213.09 W; time, X2 = 2.38 min; Ymax = 11.82%).

This Figure 4 shows a two-dimensional contour graph describing the effect of ultrasound power (x1) and time (x2) on the ergosterol (y) content in yeast.

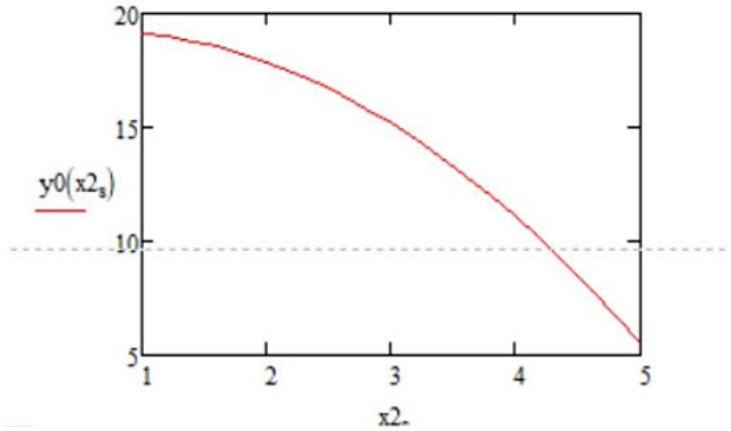



**Fig. 4.** Two-dimensional contour graph of optimization results.

9 different ultrasonic treatment modes were used in the experiment, covering a wide range of power and time parameters. A quadratic regression model was constructed using two-factor mathematical analysis. The results of the response surface analysis showed that under optimal ultrasonic treatment conditions of 213.09 W and 2.38 minutes, the ergosterol content in yeast reached the highest value of 11.82%. Proper ultrasound exposure can significantly enhance the biosynthetic ability of sterols by yeast. It can be seen from the contour graph that within the appropriate power and time range, the ergosterol content will increase with an increase in these two factors, demonstrating an obvious peak.

This result shows that ultrasound treatment at a certain power and time can significantly increase the yeast ability to biosynthesize ergosterol. This may be due to the fact that it promotes the synthesis and accumulation of ergosterol by improving the permeability of the cell wall and activating enzymes related to metabolism.

An increase in ergosterol synthesis in yeast biomass under ultrasound exposure is possible due to positive factors caused by ultrasound, such as an increase in dissolved oxygen in the substrate and an increase in the permeability of the cell wall between the inner and outer parts of the cell membrane.

Ultrasound is a periodic longitudinal wave that creates forces of different directions and intensity in any position of the yeast cell at a certain point in the vibration cycle [13-15]. The tangential forces derived from these forces cause the tangential movement of the cell membrane, making the local cell membrane thinner or the local pores larger. As a result, the permeability of the cell membrane increases, and the metabolism between the two sides of the cell membrane accelerates [15]. In addition, ultrasound can cause a change in the cell membrane potential, which leads to a change in cell membrane permeability, affecting the ability of the cell to absorb and transport nutrients [13, 15].

Compared with other studies based on changes in fermentation conditions, nutrient medium and other factors, in this experiment, ultrasound exposure was innovatively applied to optimize the synthesis and accumulation of ergosterol by yeast *Saccharomyces cerevisiae*. Compared with traditional methods of regulating ergosterol synthesis, ultrasonic treatment has the advantages of ease of operation, low energy consumption, and environmental friendliness. In the future, it is possible to further study the synergistic effect of using ultrasonic treatment under various cultivation conditions in combination with other modern

biotechnological approaches (such as cellular engineering, optimization of fermentation parameters, etc.), as well as further improvement of ergosterol production and environmental friendliness of the process.

## 4 Conclusion

In this work, the yeast *Saccharomyces cerevisiae* is taken as the object of research. Two fundamentally different exposure factors were used in the experiment, which could affect the intensity of ergosterol biosynthesis by yeast. The research results showed that the presence of a carbohydrate component in the nutrient medium has a positive effect on the rate of ergosterol synthesis, as well as the addition of sodium nitrate as an additive. An increase in the N:C ratio in favor of nitrogen reduces the ergosterol synthesis.

It has been shown that ultrasonic exposure can be effectively used to intensify the ergosterol synthesis by yeast during cultivation. 9 different ultrasonic treatment modes were used, covering a wide range of exposure power and time. Through two-factor mathematical planning and analysis of the response surface, a quadratic regression model was built. The results of the response surface analysis showed that under optimal conditions of exposure to ultrasound with a power of 213.09 W and a duration of 2.38 minutes, the ergosterol content in yeast reaches a maximum value of 11.82%. Under these conditions, the synthesis of ergosterol and the efficiency of biosynthesis were significantly increased.

This study provides theoretical foundations and practical recommendations for the efficient production of ergosterol using *Saccharomyces cerevisiae*, provides an increase in ergosterol yield, and lays the foundation for the development of a technology for an efficient and environmentally friendly process for the production of ergosterol using yeast. This is of great practical importance for the food and pharmaceutical industries.

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