BIOTECHNOLOGICAL ASPECTS CONCERNING THE ERGOSTEROL OBTAINING FROM YEASTS

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Abstract. This paper reports on the experimental results for selection of *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain with high potential for ergosterol biosynthesis; determination of morpho-cultural and physiologo-biochemical characteristics of this yeast strain; establishment of the effect of low-intensity millimeter waves on ergosterol biosynthesis. Research results contributed to the elaboration of technological flow for obtaining of high yield of ergosterol from yeasts. The significant increasing of ergosterol content is possible due to the utililization of optimized nutritive medium with manganese acetate (0.012 g/L) and glucose (40.0 g/L); the treatment of inoculum with low-intensity millimeter waves for 20-30 minutes.

Keywords: yeast, *Saccharomyces carlsbergensis*, sterols, ergosterol, culture medium, millimeter waves.

INTRODUCTION

Sterols possess a high biological activity that determines their wide application in many different fields. There is a high demand in medicine, food industry, zootechny and cosmetology for the capacity of ergosterol to transform in the vitamin D_2 (ergocalciferol). Recent biomedical studies confirm the opportunity of the utilization of ergosterol as an active part of new medical remedies with anti-rickets, anti-cancer, anti-leucemic, immunomodulatory and hematopoietic properties, as well as destined for the maintenance of cellular homeostasis and for the prevention of disorders of the human endocrine and reproductive systems [10-12, 14, 16, 25, 30, 31, 39].

Ergosterol ($C_{28}H_{44}O$) or provitamin D has been found in yeasts, other fungi and some bacteria [2, 9, 13]. Yeasts are considered high sterols producers. Ergosterol, as well as cholesterol, is present in many animal tissues and skin (where it takes place the conversion to the vitamin D_2).

Yeasts are the source of valuable bioactive substances, especially ergosterol. These microorganisms have an important role among other biotechnological tools used for the production of vitamins and also have great advantages compared to traditional sources.

Saccharomyces yeast strains have been received a great attention due to the capacity for ergosterol biosynthesis [1, 26, 34]. Thus, the wide range of sterol applications promotes not only to technological improvements that increase efficiency of their production, but also to find out new primary sources of raw material. Besides *Saccharomyces* yeast strains, *Aspergillus fumigatus* and *Candida albicans* are known as ergosterol producers [2, 15, 23].

Thereby, the scientific foundation of direction of processes of yeasts (active sterols producers) cultivation, as well as the elaboration of procedures of microbian ergosterol obtaining is important. The aim of this study was the elaboration of new technology of ergosterol (provitamin D) obtaining from yeast biomass.

MATERIALS AND METHODS

Strains. *Candida, Saccharomyces, Rhodotorula, Sporobolomyces* yeast strains deposited in National Collection of Non-Pathogenic Microorganisms of the Institute of Microbiology and Biotehnology of Academy of Sciences of Moldova have been studied. Yeast strains *Saccharomyces cerevisiae* CNMN-Y-17, *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 were isolated from wine sediments. *Saccharomyces carlsbergensis* CNMN-Y-15 - brewer's yeast strain. *Saccharomyces cerevisiae* CNMN-Y-16 and *Saccharomyces cerevisiae* CNMN-Y-11 - baker's yeast strains.

The determination of morphological and physiological parameters were effectuated according to [5, 18].

Medium. The following medium for were used in this study: seed culture medium with beer wort, YPD, (g/L): glucose - 20.0, peptone – 20.0, yeast extract – 10.0; fermentation medium, (g/L): $(NH_4)_2SO_4$ – 1.0, K_2HPO_4 – 2.0, $MgSO_4$ – 1.0, yeast autolysate – 10.0 [4]; fermentation medium Shang, (g/L): industrial glucose – 60.0, corn steep liquor – 15.0, KH_2PO_4 – 6.0, $MgSO_4$ – 3.0, $CuSO_4 \cdot 5H_2O$ – 1.0, $FeSO_4 \bullet 7H_2O$ – 1.0 and $ZnSO_4 \cdot 7H_2O$ – 10.0 [29]; fermentation medium Rieder, (g/L): glucose – 30.0, $(NH_4)_2SO_4$ – 3.0, $MgSO_4 \bullet 7H_2O$ - 0.7, NaCl - 0.5, $Ca(NO_3)_2$ - 0.4, KH_2PO_4 – 1.0, yeast autolysate – 10.0, pH- 5.0-6.0 [3].

Medium of culture MN-S with the following composition (g/L): $(NH_4)_2SO_4$ – 1.0; K_2HPO_4 – 2.0; $MgSO_4$ – 1.0; yeast autolysate – 10.0; glucose – 40.0; manganese acetate – 0.012, pH-5.5 had been elaborated [21].

Culture conditions. Cultivation was carried out in Erlenmayer 1 L flasks containing 0.2 L of the culture medium at 200 rpm. agitation rate, 25±1ºC, with air flow rate 7-8 mg/L for 96 hours.

Dry cell weight was determined gravimetrically [17].

Ergosterol extraction was effectuated according to [33].

Lipid extraction from biomass was performed by the method Bligh, Dyer [17] and by the new procedure in our modification [32].

The optimization of nutritive medium was effectuated by methods of mathematical plannig of experiments [20]. The optimization is based on the utilization of two experimental factors: carbon source (the glucose) and precursor of ergosterol (manganese acetate). The optimization of culture medium for the obtaining of maximum content of ergosterol has been carried out in some consecutive stages: the experience according to plan „Fractional factorial experiment ($FFE2^2$)" with the determination of direction of variation of concentrations (increase or decrease) and the experiment according to plan „Movement along the gradient" during which the most effective combination of the essential and nonsential factors has been selected.

Low - intensity millimeter waves. As generator of low-intensity millimeter waves (power flux density $mW/cm^2 \div 10mW/cm^2$) the device "Явь-1" (Russian Federation) was used, where the lengh λ=5.6 mm (53.8 GHz). Yeast material, grown on a solid medium (agarized beer wort) at 25°C during 48h was subjected to treatment with millimeter waves (periodic mode). The irradiated cells were transferred into liquid medium for the obtaining of inoculum.

Statistical analysis: Results were expressed as the mean±standard deviation (SD). Statistical evaluations were performed using *t* test. A value of $P<0.05$ was considered significant. The results were performed according to Student's t-test.

RESULTS

Screening of yeast strains

Recent studies of biosynthetic potential of *Saccharomyces*, *Candida* şi *Rhodotorula* yeast strains cultivated in the presence of beer wort have demonstrated the increased activity of oleogene components. A high content of ergosterol at brewer's and baker's yeast has been detected. The maximum content of ergosterol – 22.65% of total lipids, was registered at *Saccharomyces carlsbergensis* CNMN-Y-15. *Saccharomyces cerevisiae* CNMN-Y-16 and *Saccharomyces cerevisiae* CNMN-Y-11 contained about 22.01 – 21.45% ergosterol of total lipids (Table 1). Yeast strains *Saccharomyces cerevisiae* CNMN-Y-17, *Saccharomyces cerevisiae* CNMN-Y-18 and *Saccharomyces cerevisiae* CNMN-Y-19 are characterized by insignificant content of lipids and ergosterol. *Candida* and *Rhodotorula* yeast strains have high lipids content; at the same time, the content of ergosterol did not exceed that of brewer's or baker's yeast (Table 1).

The analysis of results has demonstrated linear dependency between dry cell weight and ergosterol content at *Saccharomyces* yeast strain. The correlation coefficient is positive r = + 0.846. This dependency is insignificant for *Candida* and *Rhodotorula* strains: r = + 0.13 and r = - 0.11 respectively. The obtained results can serve as the base for the elaboration of some efficient procedures of direction of ergosterol biosynthesis at yeasts. The comparative analysis of obtained results of screening of yeast strains with

Table 1. Dry cell weight, lipids and ergosterol contents at selected yeast strains

No.	Yeast strain	Dry cell weight, g/L	Lipids, %	Ergosterol, % of total lipids
Class *HEMIASCOMYCETES*				
1.	*Saccharomyces cerevisiae* CNMN-Y-11	6.39±0.30	10.93±0.71	21.45±0.7
2.	*Saccharomyces cerevisiae* CNMN-Y-16	7.47±0.20	9.0±0.01	22.01±0.1
3.	*Saccharomyces carlsbergensis* CNMN-Y-15	6.33±0.18	12.50±0.30	22.65±0.42
4.	*Saccharomyces cerevisiae* CNMN-Y-18	3.76±0.12	5.55±0.11	11.59±0.11
5.	*Saccharomyces cerevisiae* CNMN-Y-17	4.84±0.15	6.16±0.10	15.60±0.38
6.	*Saccharomyces cerevisiae* CNMN-Y-19	4.00±0.14	4.06±0.12	13.10±0.27
Class *BLASTOMYCES*				
7.	*Candida tropicalis* CNMN-Y-21-303	5.74±0.71	16.96±0.22	10.33±0.73
8.	*Candida utilis* CNMN-Y-22 -74	5.86±0.05	15.56±0.48	10.87±0.47
9.	*Candida utilis* CNMN-Y-24-322	6.27±0.65	13.76±0.92	13.46±0.56
10.	*Candida rugosa* CNMN-Y–23-67	3.98±0.69	10.06±0.81	8.25±0.33
11.	*Candida albicans* CNMN-Y-25	5.78±0.17	12.17±0.66	8.60±0.12
12.	*Candida diddensis* CNMN-Y-26-1446	6.22±0.36	21.70±1.93	8.92±0.27
13.	*Candida pelliculosa* CNMN-Y-27-01	11.68±0.84	25.97±1.52	7.92±0.43
14.	*Rhodotorula glutinis* CNMN-Y-08	2.87±0.98	24.89±1.25	7.65±0.41
15.	*Rhodotorula rubra* CNMN-Y-09	2.91±0.43	21.86±1.35	7.98±0.40
16.	*Rhodotorula mucilaginosa* CNMN-Y-10	5.14±0.71	16.90±0.40	7.88±0.49
17.	*Rhodotorula gracilis* CNMN-Y-1/3	10.09±1.15	18.69±0.92	7.39±0.46
18.	*Rhodotorula gracilis* CNMN-Y-1/4-04	9.12±0.80	18.67±0.30	7.6±0.34
19.	*Rhodotorula gracilis* CNMN-Y-I/15	8.56±0.38	20.52±1.53	7.47±0.29
20.	*Rhodotorula gracilis* CNMN-Y-II/6	8.56±0.68	20.06±1.09	8.51±0.86
21.	*Rhodotorula gracilis* CNMN-Y-II/9	7.94±0.81	17.97±0.17	13.14±0.88
22.	*Rhodotorula gracilis* CNMN-Y-III/5-05	11.07±0.89	22.10±1.10	9.21±0.96
23.	*Rhodotorula gracilis* CNMN-Y-III/20-06	10.76±0.87	23.59±1.54	8.51±0.13
24.	*Rhodotorula gracilis* CNMN-Y-IV/14	8.85±0.26	21.04±1.96	9.07±0.33
25.	*Rhodotorula gracilis* CNMN-Y-V/12	10.22±0.42	15.14±0.47	7.54±0.31

scientific literature [13] allowed the conclusion that *Saccharomyces carlsbergensis* CNMN-Y-15 is important biotechnological tool for ergosterol obtaining.

Biosynthetic potential is associated with metabolic flexibility. The profound study of biological peculiarities of microorganisms contributes to the identification of the mode of direction of processes. In this context, it is important to establish morpho-cultural and physiologo-biochemical properties of yeast strain selected as bioactive substances producer.

The study of *Saccharomyces carlsbergensis* CNMN-Y-15 has demonstrated that cells have cylindrical or ellipsoidal form, are single or in pairs and rarely form agglomerations. The size of cells is typically 5-10 μ in diameter. Can multiply either asexually by vegetative multiplication by budding or sexually by forming ascospores. Sometimes, this yeast strain can form pseudohyphae. The type of respiration is aerobic. Yeast strain can form sediment in liquid culture medium.

Among physiologo-biochemical properties the fermentation is the most important. *Saccharomyces carlsbergensis* CNMN-Y-15 does not assimilate nitrates and urea. The yeast metabolizes glucose, sucrose**,** melibiose (a distinguishing feature among brewer's and baker's yeast), mannose, maltose, less arabinose and rhamnose and does not assimilate lactose. The strain is classified as tolerant to the acidity; optimal pH is 4.5-5.5, optimum growth temperatures in the range from 25 to 27^0C.

The selected yeast strain synthesized 3.8-3.9% of ergosterol per dry biomass at submerged cultivation on YPD or Rieder medium. The ability to synthesize high content of ergosterol and morpho-cultural and physiologo-biochemical properties of selected strain have allowed to patent *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain as important biotechnological tool [35].

Statistical optimization of culture medium

It was important to select an optimal nutritive medium with balanced chemical composition with the aim to obtain high yeast productivity. In this context, investigations of the influence of some carbon and nitrogen sources, precursors and other factors that regulate ergosterol biosynthesis have been previously effectuated and optimal variants have been selected [22, 36]. According to the obtained results, glucose and manganese acetate were proposed for the following investigation.

Statistical and regression analysis of an monofactorial experiment with all components of culture medium was performed. Previously, two essential factors: glucose and manganese acetate [$Mn(CH_3COO)_2 \cdot 4H_2O$] that can significantly influence sterols biosynthesis were detected [21]. Results of this study were used for mathematical optimization of culture medium with the goal to obtain maximum content of ergosterol.

The culture medium recommended by Anghel [4] served as control. Using this medium *Saccharomyces carlsbergensis* CNMN-Y-15 accumulated 1.28±0.01 g/L dry cell weight, that contained 7.3±0.51% of ergosterol.

The optimization of culture medium for the obtaining of maximum content of ergosterol has been carried out in some consecutive stages: the experience according to plan „Fractional factorial experiment ($FFE2^2$)" with the determination of direction of variation of concentrations (increase or decrease) and the experiment according to plan „Movement along the gradient" during which the most effective combination of the essential and nonsential factors was detemined.

Initially, plan for fractional factorial experiment $EFF2^2$ was elaborated (Table 2). In accordance with the obtained results, regression coefficients were calculated by algorithm Iets [20]. The regression equation took the form: $\mathbf{Y=10.91 + 0.93\ X_1 + 0.10\ X_2 - 0.18\ X_1X_2}$.

Further, the direction of modification of compounds concentrations – to increase or decrease has been detected. Results of the experiment „Movement along the gradient" are presented in table 3 below (Table 3).

The utilization of this medium for the cultivation of *Saccharomyces carlsbergensis* CNMN-Y-15 allowed the obtaining up to 13.20% ergosterol per dry biomass compared to control – 7.3% ergosterol per dry biomass (increasing with 80.82%). By cultivation of the yeasts in medium with 35.0 g/L glucose and 0.011 g/L manganese acetate, the ergosterol content per dry biomass was 12.22% (increasing with 80.82% compared to the control) (Fig. 1).

Thus, new nutritive medium – **MN-S** with the following composition (g/L): $(NH_4)_2SO_4$ – 1.0; K_2HPO_4 – 2.0; $MgSO_4$ – 1.0; yeast autolysate – 10.0; glucose – 40.0; manganese acetate – 0.012, pH-5.5 has been elaborated.

Table 2. Plan for fractional factorial experiment $EFF2^2$

Factors	Glucose	Manganese acetate	Sterols, % of dry biomass	Regression coefficient (b_i)
	X_1	X_2		
Lower level (-)	30.0	5.0		
Upper level (+)	50.0	15.0		
Concentration	g/L	mg/L		
1	-	-	9.78	10.91
2	+	-	12.02	0.93
3	-	+	10.18	0.10
4	+	+	11.69	-0.18

Table 3. Plan of the experiment „Movement along the gradient"

Value	Factors		Dry cell weight, g/L	Ergosterol, % per dry biomass
	Glucose	Manganese Acetate		
b(i)	0.93	0.10		
λ(i)	10	5		
b(i) · λ(i)	9.30	0.5		
H(i)	5	1		
Concentration	g/L	mg/L		
1	30.0	10.0	5.12	10.74
2	35.0	11.0	5.10	12.22
3	**40.0**	**12.0**	5.00	**13.20**
4	45.0	13.0	5.08	12.08
5	50.0	14.0	5.52	11.63
6	55.0	15.0	5.58	10.56

Note: *b(i)* – regression coefficient; *λ(i)* – units of variation; *H(i)* – step.

Biological effect of low-intensity millimeter waves

It was established by different scientists that low-intensity that low-intensity millimeter waves stimulated biosynthetic activity of yeast, proven by the change of mode of carbon sources assimilation, in particular, pathway of maltose fermentation. These modifications contribute to the intensification of fermentation processes and reduction of biotechnological cycle. The results reported in literature have demonstrated that low-intensity millimeter waves can serve as regulator of synthesis of valuable bioactive substances [6].

During the investigations of the influence of low-intensity millimeter waves on ergosterol biosynthesis at *Saccharomyces carlsbergensis* CNMN-Y-15 cultivated on YPD medium it was found that biological effect of low intensity millimeter wave action on the yeast investigated at the population and cellular level may cause stimulation or inhibition of biological activity or may remain neutral, fact that depends on the regime and duration of treatment, phase of population formation, and number of treatments with millimeter waves. The obtained results were used for the elaboration of processes of increasing of the productivity of the yeast *Saccharomyces carlsbergensis* CNMN-Y-15 and contents of bioactive substances in the biomass with the utilization of low intensity millimeter waves as a stimulating factor [37, 38].

The influence of low intensity millimeter waves on the some biological objects is intensively studied during the last years. An essential factor of the low-intensity millimeter waves action on the yeast population it is the duration of treatment. It was previously established by the studying of the biological effect of low intensity millimeter waves in the time of 5, 10, 15, 20, 30 minutes that the time of irradiation is important (Fig. 2). The study of the influence of the mode of low-intensity millimeter waves treatment has revealed the optimal time of irradiation 20-30 minutes.

The results obtained during experiments with *Saccharomyces carlsbergensis* CNMN-Y-15 cultivated in the optimized medium MN-S or in the medium Shang, confirmed that the treatment of with low intensity millimeter waves stimulated ergosterol biosynthesis. These fermentation mediums can be distinguished by the composition**,** especially, by the presence of manganese acetate in medium MN-S.

The optimal time of irradiation was 30 minutes. MN-S and Shang mediums without irradiation were used as controls. The irradiation of yeast strain in the mentioned time increases the content of ergosterol with 38.9 and 21.4% compared to control and contributes to the biomass accumulation with 27.4 and 19.6% (Table 4).

The technology of ergosterol obtaining from yeast

Intensive depelopment of modern biotechnology contributes to the improvement of procedures of ergosterol extraction from yeast biomass. There are a number of well-known procedures of direct ergosterol extration from biomass or based on preliminary lipids extraction [9, 29]. These procedures have some disadvantages as the difficulty of performing, long length of extraction and large consumption of reagents.

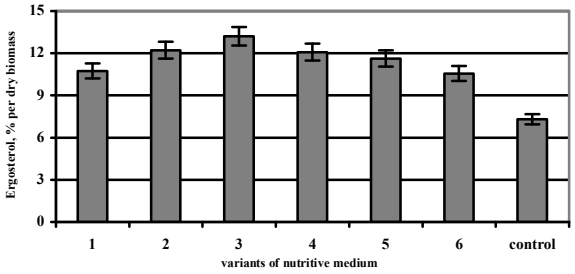

Figure 1. Dry cell weight of *Saccharomyces carlsbergensis* CNMN-Y-15 and ergosterol content in biomass at the cultivation in the presence of the glucose and manganese acetate

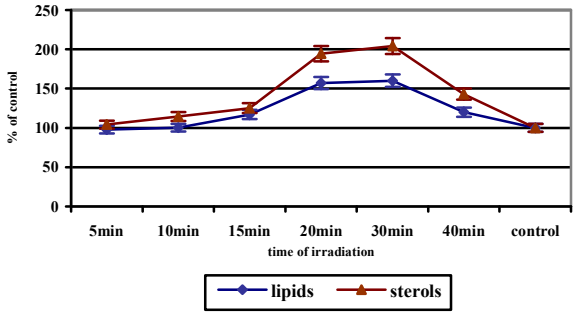

Figure 2. The influence of time of irradiation of low-intensity millimeter waves on lipids and sterols content at *Saccharomyces carlsbergensis* CNMN-Y-15

The essence of elaborated technological process lies in fact that saponification process has no effect on sterols and they were extracted easily from the alkaline solutions with organic dissolvers.

The new optimized procedure of extraction consists of the following stages: the saponification of lipids from freeze-dryeed biomass with sodium hydroxide on the water bath; the treatment of obtained extract after the cooling with isopropyl alcohol; periodic agitation for 30-60 minutes; the separation of non-saponified extract from ergosterol and alcohol (the upper stratum); removal of solvents by distillation using vacuum vaporizer; determination of weight of dry ergosterol. The procedure is characterized by initial freezing of yeast biomass for 30-60 minutes at temperature -18^0 C, the saponification of biomass with the mix that contained sodium hydroxide, water and isopropyl alcohol in the report 25:50:25 on water bath, at temperature 75^0 C for 30 – 60 minutes.

The foolowing procedure characterized by the treatment of yeast biomass with the mix that contained sodium hydroxide, water and isopropyl alcohol in the report 40:20:40 was used as control [19].

Ergosterol extracted according to elaborated procedure 1-2 constituted of 2.26 up to 2.52% per dry biomass which respectively surpasses with 18.32 and 31.93% the content obtained in proximate conditions, the duration of the procedure is 3 hour less than control (Table 5). The procedure 1-2 of ergosterol extraction from yeast biomass was patented [33].

The analysis of the results have demonstrated that the new optimized technology for ergosterol obtaining was possible due to the efficient screening of the yeast strains with high and stable potential of ergosterol biosynthesis; the optimization of chemical composition of nutritive medium by the procedures of directed synthesis with the utilization of low - intensity millimeter waves as regulators of sterol contents.

The proposed technology is based on the utilization of *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain – active producer of ergosterol, optimized nutritive medium MN-S with equilibrated composition, optimum mode of low - intensity millimeter waves application. The technological scheme that assures 700 mg/L total yield of ergosterol is presented below (Fig. 3).

DISCUSSIONS

Investigations referring to the microbial ergosterol obtaining take place in some countries [15]. The perspective of microbiological synthesis of sterols consist in the fact that the utilization of specialized yeast strains as active producers of ergosterol that enhanced the choice of raw material for obtaining of natural sterols preparations with the large application. It is evident the importance of investigations referring to the screening of producers with high content of sterols, the optimization of culture medium, the elaboration of procedures of obtaining of bioactive substances that contributes to the production of biologically pure preparations destined to improve the health of the population**.** The screening of yeast strains has demonstrated the different levels of lipids and ergosterol content. *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain presents great biotechnological interes as producer of ergosterol.

This study study reveals concerning data on new extraction procedure of yeast ergosterol produced under optimized processes. The new optimized procedure of ergosterol extraction from yeast biomass contributes to the increasing of ergosterol content up to 18.3...31.9% with reduction in duration of procedure.

Over the past decades, numerous experimental researches were effectuated to evaluate the effect of metabolic precursors and some carbon and nitrogen sources on yeast strains [24]. According to the obtained results, raffinose, sucrose and glucose contribute to the significant increase of sterols content (with 40-50%) [8]. The obtained results confirm the conception advanced in 1954 by [27], that acetate is the basic precursor in ergosterol biosynthesis. Transition metal ions have a great importance for the assimilation of nutrient substrate or for the regulation of biosynthetic activity of yeasts as cofactors of different enzymes. Mn^{2+} is known to catalyze the activity of Acetyl-CoA carboxylase that is required for sterols biosynthesis [7]. The *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain cultivation on optimized medium MN-S with glucose (40.0 g/L) and manganese acetate (0.012 g/L) assures up to 13.2 % ergosterol per dry biomass.

Table 4. The influence of low-intensity millimeter waves on biosynthetic capacity of *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain (30 minutes of irradiation)

Medium of cultivation	Dry cell weight, g/L	% Control	Ergosterol, % per dry biomass	% Control
MN-S + irradiation	5.11±0.20	27.4	14.67±0.22	38.9
Shang + irradiation	7.61±0.27	19.6	11.67±0.84	21.4

Table 5. The efficiency of the procedures of ergosterol extraction from *Saccharomyces* yeast strain biomass

No.	Procedure of extraction	Duration of the saponification	Ergosterol, % per dry biomass	% Control
1.	Control	4 hours	1.91±0.01	100.00
2.	Procedure 1	30 minutes	2.26±0.04	118.32
3.	Procedure 2	60 minutes	2.52±0.02	131.93

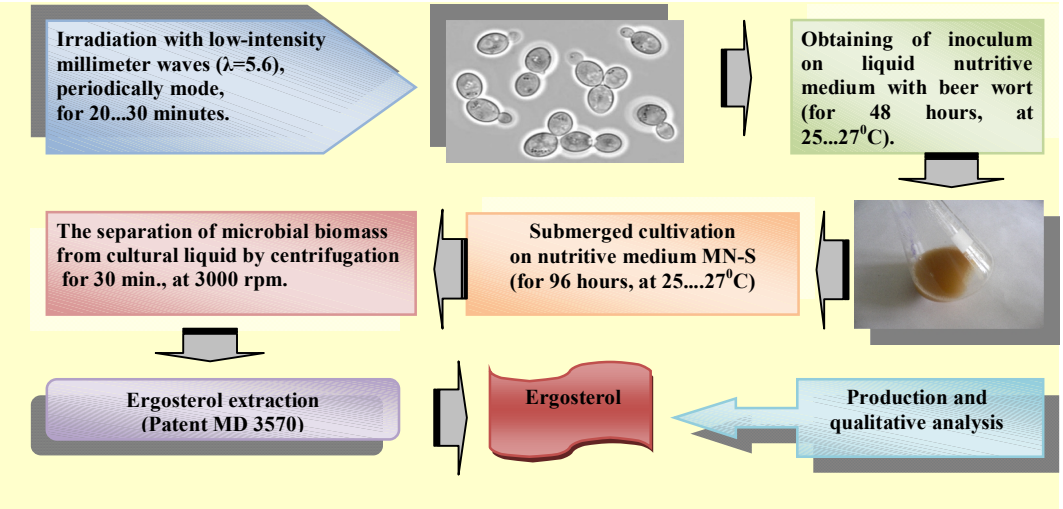

Figure 3. The technological scheme of obtaining of ergosterol from biomass of *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain

The influence of low-intensity millimeter waves on cellular metabolism presents a new direction of biotechnology of yeasts cultivation. It has been established that low-intensity millimeter waves contribute to the obtaining of high ergosterol content in the biomass of yeasts. The formation of superoxid radical and peroxide of hydrogen takes place during the irradiation [28]. The formed active radicals break the activity of oxidase enzymatic complex forming intermediate products that take part in sterol biosynthesis. The treatment of inoculum with low-intensity millimeter waves for 20-30 minutes stimulates the process of biomass accumulation and ergosterol biosynthesis.

The optimized technology with high yield of microbial ergosterol has been elaborated as the result of the screening of active yeast strain, the utilization of new nutritive medium, combination of precursors and inductors of sterols biosynthesis, optimal parameters of application of millimeter waves of low intensity and the new procedure of extraction of sterols.

The total yield of ergosterol of 700 mg/L was obtained as the result of optimized process of directed cultivation of *Saccharomyces carlsbergensis* CNMN-Y-15 yeast strain utilization that would create the new perspective for production of preparations with vitamin D activity.

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