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The potential of corn oil cake hydrolysate application as a substrate for *Saccharomyces cerevisiae* cultivation

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ABSTRACT

Background: The yeast *Saccharomyces cerevisiae* is a source of biologically active substances, which can be used in the development of functional food products. This drives the search for inexpensive, nutrient-rich substrates for the cultivation of this yeast. Such substrates can be based on agricultural by-products such as corn oil cake (COC). It is of particular interest due to its chemical composition, including high protein content (up to 25%), lipids (up to 16%), and carbohydrates (up to 42%).

Objective: This study aimed to evaluate the potential of using COC as a raw material for producing a substrate for the *S. cerevisiae* cultivation.

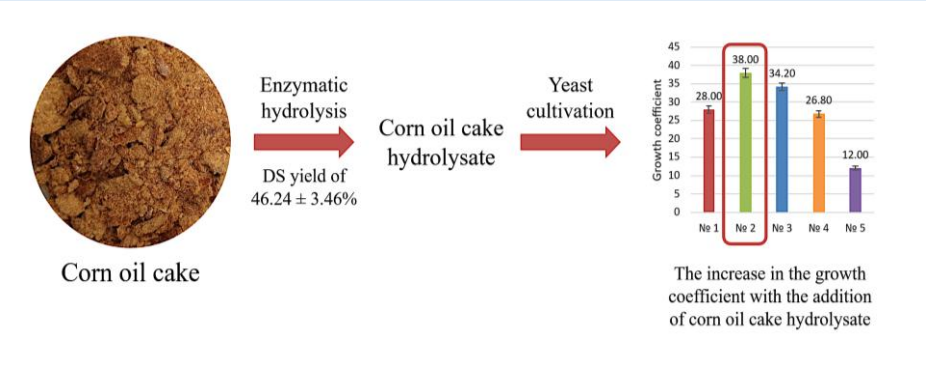
Methods: During the study, the chemical composition of COC was investigated by the AOAC methods. Enzymatic hydrolysis of COC was performed at a mash ratio of 1:10; at a temperature of 75°C for the first four hours and 60°C for the following four hours; and stirring at 500 rpm. After the completion of enzymatic hydrolysis, the hydrolysate was separated using a hydraulic press. Batch cultivation of *S. cerevisiae* L1 was performed at an initial concentration of dissolved solids of 6%, a temperature of 28 °C, for 24 hours.

Results: The COC had the following chemical composition: moisture – 4.74 ± 0.01 %; crude protein – 22.87 ± 0.58 % on a dry matter (DM) basis; lipids – 14.99 ± 0.47 % on a DM basis; crude fiber – 9.9 ± 1.4 % on a DM basis; and ash – 3.52 ± 0.51% on a DM basis. It has been shown that enzymatic hydrolysis of COC provides a dissolved solids (DS) yield of 46.24

± 3.46% according to the mode proposed in this study. The use of COC hydrolysate as an additive to malt wort increased the efficiency of the cultivation process. Thus, at a COC hydrolysate concentration of 25% in the substrate composition, an increase in the growth coefficient and biomass yield was observed by 10.0 and 4.21%, respectively.

Conclusion: As a result of the work, it was demonstrated that the COC hydrolysate can be used as a substrate for the cultivation of *S. cerevisiae*. At the same time, when COC hydrolysate is used as an additive to malt wort, the efficiency of the *S. cerevisiae* cultivation process can be increased.

Key words: corn oil cake, enzymatic hydrolysis, yeast cultivation, *Saccharomyces cerevisiae*



Graphical Abstract: The potential of corn oil cake hydrolysate application as a substrate for Saccharomyces cerevisiae cultivation.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is used in numerous biotechnological processes [1]. It has a long history of application in food technology for producing fermented foods, including alcoholic beverages (beer, wine, cider, and hard liquors) [2], and bread and bakery products [3-4]. *S. cerevisiae* can also be used to obtain functional food components and bioactive compounds such as β-glucans and mannoproteins [5]. Recently, cultures of this yeast species have been employed to produce microbial protein (single-cell protein) [6], which can subsequently be used to enrich food and animal feed [7]. Additionally, *S. cerevisiae* is implemented in the pharmaceutical industry to produce insulin analogues and therapeutic proteins (e.g., albumin and antithrombin) [8]. *S. cerevisiae* is also considered one of the main yeast species used to produce biofuels and in bioremediation [9-10].

These applications require biomass production, and the cultivation process should be efficient and economically feasible. This can be achieved by applying secondary material resources of the agro-industrial sector or their processed products as a substrate.

For many years, molasses has been widely used as a by-product for yeast cultivation, due to its large production volume [11] and chemical composition, which is characterized by a high content of simple sugars, mainly sucrose, reaching up to 67.3% [12]. However, when molasses is used as a substrate, it is required to add vitamins (biotin and pantothenic acid) as well as copper and zinc salts for yeast metabolism [13-15].

Interest has grown recently in using starch-containing and lignocellulose hydrolysates from agricultural by-products for the cultivation of *S. cerevisiae* [16]. It has been shown that waste from starch and oilseed production, as well as lignocellulose agricultural waste, can form the basis of the nutrient medium [17].

Although corn is one of the most widely cultivated grain crops [18], there are few reports on using its processing by-products (bran, cake) as substrates for yeast cultivation. Among the corn processing by-products, COC is particularly noteworthy. It is obtained after oil extraction from corn germ separated during grain processing [19]. The COC chemical composition is notable for a high protein, lipid, and starch content – up to 36.50% [20], 15.51% [21], and 42.2% [22], respectively.

Thus, the aim of the research was to evaluate the potential of using COC as a raw material for preparing a substrate for the *S. cerevisiae* yeast cultivation.

MATERIALS AND METHODS

The object of the study: The object of the study was COC (LLC "TSP", Russia).

Investigation of the chemical composition of COC: The fractional composition of COC was determined by sieving through a set of sieves (Stroyuniversal, Russia) with mesh sizes ranging from 0.125 mm to 1.250 mm, followed by weighing each fraction. The COC was ground using a VLM-6 cutting mill (Vilitek, Russia).

The chemical composition of COC was analyzed by measuring the following parameters: moisture (method 930.15, AOAC, 1990); crude protein (method 976.05, AOAC, 1990) using an automatic nitrogen analyzer UDK 159 (VELP Scientifica, Italy); lipids (method 920.39, AOAC, 2012); crude fiber (method 962.09, AOAC, 1990); and ash (method 942.05, AOAC, 2005). Starch content was calculated by difference.

Conducting enzymatic hydrolysis: The following enzyme preparations were used for hydrolysis: SEBPro X, 50 mg/100 g (85000 TU/g; Advanced Enzyme Technologies Ltd, India); AMYLEX 5T, 20 mg/100 g (min. 13775 AAU/g; Danisco, Denmark); LAMINEX MaxFlow 4G, 40 mg/100 g (min. 13680 XBU/g, min. 9090 BBU/g; Danisco, Denmark); and Cellulase, 40 µl/100 g (4000 units/ml; Biopreparat Trading House, Russia) (Table 1).

Table 1. Optimum temperature and pH for enzyme preparations.

Enzyme preparation	SEBPro X	AMYLEX 5T	LAMINEX MaxFlow 4G	Cellulase
Temperature optimum, °C	60-85	70-90	60-80	50-65
Optimum pH	6.0-8.0	4.5-7.5	5.0-6.5	3.5-5.0

Enzymatic hydrolysis was performed in mash metal cups with constant stirring using an RZR 2020 overhead stirrer (Heidolph, Germany). Temperature was maintained using a LOIP LB-163 water bath (LOIP CJSC, Russia).

To determine the composition of the enzyme preparation complex, hydrolysis was performed under two different conditions. The first (Mode 1.1) was performed without Cellulase at a constant temperature of 75 °C and pH of 5.5 using SEBPro X, AMYLEX 5T, and LAMINEX MaxFlow 4G. The second (Mode 1.2) was conducted at a constant temperature of 60 °C and pH of 5.0 with the addition of all four enzyme preparations (Cellulase, SEBPro X, AMYLEX 5T, and LAMINEX MaxFlow 4G).

To evaluate the effectiveness of enzymatic hydrolysis, two modes were developed, each characterized by different temperature pauses (Table 2), reflecting the temperature optimum of the enzymes used.

Table 2. Parameters of enzymatic hydrolysis modes.

Parameter	Mode 2.1	Mode 2.2
Duration, h	8	4 → 4
Temperature, °C	60	75 → 60
Stirring, rpm	500	
pH	5.0	5.5 → 5.0
Mash ratio	1:10	
Enzyme preparations and their dosage, mg /100 g	SEBPro X (50) + AMYLEX 5T (20) +LAMINEX MaxFlow 4G (100) +Cellulase (35 mcl)	

Mode 2.1 was characterized by the constant temperature of 60 °C, with all enzyme preparations added simultaneously at the start of the process. Mode 2.2 included two temperature pauses at 75 and 60 °C. At the start of the first pause, SEBPro X, AMYLEX 5T, and LAMINEX MaxFlow 4G were added; at the beginning of the second pause, after lowering the temperature to 60 ° C, Cellulase was added.

To study the dynamics of enzymatic hydrolysis, the concentration of dissolved solids (DS) was measured hourly using an automatic PTR 46 refractometer (Index Instruments Ltd., Great Britain).

After hydrolysis was finished, the hydrolysate was pressed using a jack hydraulic press (LLC "MOORE", Russia). The liquid fraction of the COC hydrolysate was also separated by centrifugation for ten minutes at 4600 rpm on a ROTANTA 460 centrifuge (Hettich, Germany).

The efficiency of hydrolysis modes was evaluated according to the following parameters: DS yield rate (Formula 1) and DS yield (Formula 2) during the hydrolysis.

$$v = \frac{C_2 - C_1}{t}, \#(1)$$

where v — DS yield rate, °Bx/h; C_2 — concentration of DS in the liquid fraction at the end of hydrolysis, °Bx; C_1 — concentration of DS in the liquid fraction at the beginning of hydrolysis, °Bx; and t — duration of hydrolysis, h.

$$\varphi = \frac{V \cdot C}{m \cdot (1 - k)} \cdot 100\ \%, \#(2)$$

where φ — DS yield, %; V — volume of separated liquid fraction, ml; C — concentration of DS in the liquid fraction of hydrolysate, %; m — the weight of the sample of COC, g; and k — moisture content of COC, %.

The analysis of the separated liquid fraction of the hydrolysate was conducted by determining the free amine nitrogen (FAN) concentration and the substrate consumption degree. The FAN concentration was measured using the ninhydrin method [23]. The substrate consumption degree achieved by liquid-phase batch cultivation over 24 hours was calculated as follows (Formula 3):

$$DSC = \frac{C_1 - C_2}{C_1}, \#(3)$$

where DSC — the substrate consumption degree, %; C_1 — concentration of DS at the beginning of cultivation, %; and C_2 — concentration of DS at the end of cultivation, %.

Malt wort prepared using malt concentrate (Domashnaya Manufaktura, Russia) was used as the control substrate.

Cultivation of S. cerevisiae: In this study, we performed batch cultivation of the strain *S. cerevisiae* L1 (FGANU NIIHP, Russia) to assess the effectiveness of using COC hydrolysate as a substrate, using the malt wort as the control medium. Nutrient media with varying ratios of COC hydrolysate and malt wort were prepared, increasing the proportion of COC hydrolysate from 0% to 100% in the substrate composition in 25% increments.

Cultivation was performed in 500 ml shake flasks containing a medium volume equal to 30% of the flask capacity. The cultures were incubated at a temperature of 28 °C for 24 hours with constant stirring at 90 rpm. The

initial cell concentration was set at one million cells/ml, and the initial concentration of DS was 6.0 °Bx.

At the beginning and end of the cultivation process, the concentration of DS and dry biomass (DB) was measured. The concentration of DS was determined by the previously described method, while DB was determined by drying in a drying oven at 105 °C.

The efficiency of cultivation was assessed using the growth coefficient (Formula 4) and the biomass yield (Formula 5).

$$GC = \frac{DB_2}{DB_1}, \#(4)$$

where GC — the growth coefficient; DB_1 — DB at the beginning of cultivation, g/l; and DB_2 — DB at the end of cultivation, g/l.

$$BY = \frac{DB_2 - DB_1}{C_1 - C_2} \cdot 100\ \%, \#(5)$$

where BY — the biomass yield, %; DB_1 — DB at the beginning of cultivation, g/l; DB_2 — DB at the end of cultivation, g/l; C_1 — concentration of DS at the beginning of cultivation, %; and C_2 — concentration of DS at the end of cultivation, %.

RESULTS AND DISCUSSION

The chemical composition of COC: It has been found that the fractional composition of COC is heterogeneous, with a dominant coarse fraction with a particle diameter greater than 1.250 mm (Figure 1). The COC was ground to improve the reproducibility of the results and the effectiveness of enzymatic hydrolysis by increasing the contact area during enzymatic treatment. After grinding, the fractional composition was characterized by a prevalence of three fractions with the particle diameters of 0.250–0.500 mm, 0.500–1.000 mm, and 0.125–0.250 mm (Figure 1).

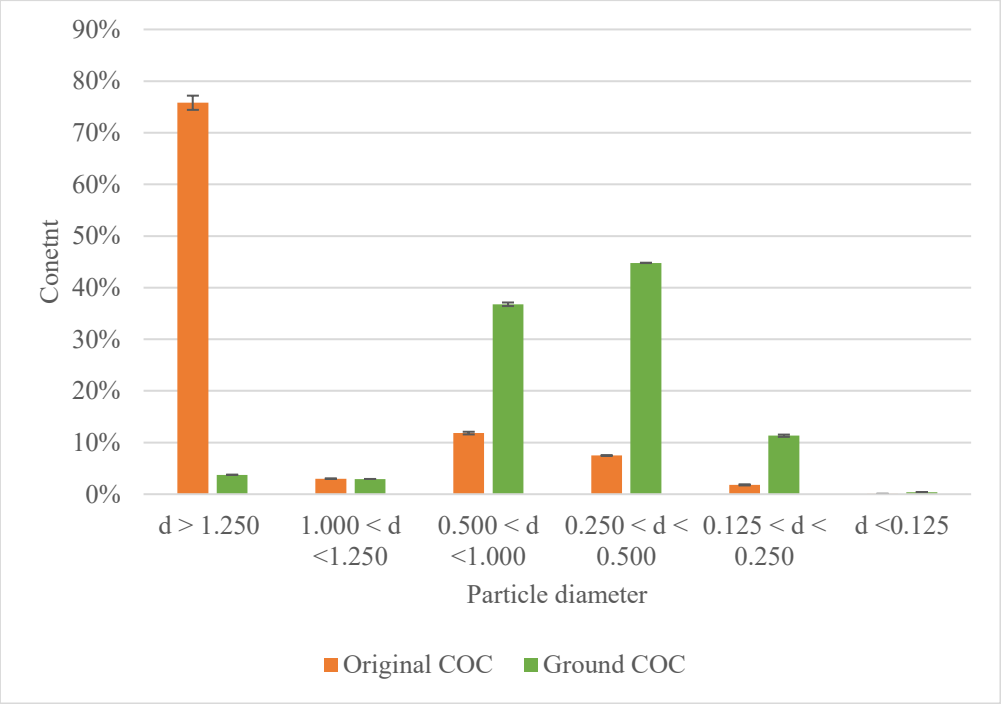


Figure 1. Fractional composition of the original and ground COC.

The chemical composition of the hydrolyzed raw materials is particularly important for developing enzymatic hydrolysis mode; therefore, the chemical composition of COC was determined. According to the results (Table 3), COC is characterized by high protein and fat contents, at 22.87 ± 0.58% and 14.99 ± 0.47% on a DM basis, respectively. Due to its high protein content, the COC hydrolysate can presumably be considered as a source of nitrogen forms digestible by yeast (amino acids and short peptides). In addition, COC hydrolysate can

serve as a source of simple sugars for yeast nutrition, due to the high concentrations of starch and crude fiber, which can be hydrolyzed by enzymes. The crude ash content of COC is quite low; however, according to some research, the primary minerals present are phosphorus and calcium [17], which are essential macronutrients for yeast growth.

Table 3. Chemical composition of COC.

Parameter	Value
Moisture, %	4.74 ± 0.01
Crude protein, % on a DM basis	22.87 ± 0.58
Lipids, % on a DM basis	14.99 ± 0.47
Crude fiber, % on a DM basis	9.9 ± 1.4
Ash, % on a DM basis	3.52 ± 0.51
Starch, % on a DM basis	48.72

Enzymatic hydrolysis of COC: When selecting enzyme preparations for hydrolysis, it was noted that the temperature optimum of Cellulase is significantly lower than that of other enzyme preparations (SEBPro X, AMYLEX 5T, and LAMINEX MaxFlow 4G), which may limit hydrolysis efficiency under a single-temperature regime.

The study included two modes of enzymatic hydrolysis: one without Cellulase (Mode 1.1) and one with it (Mode 1.2). The use of Cellulase resulted in an almost threefold increase in DS yield to 18.56 ± 2.54% (Figure 2). This may be attributed to the cellulolytic action of the enzyme, which reduces matrix rigidity and increases the volume of the separated liquid fraction. This also improves mixing efficiency, thereby enhancing the contact area between the raw material and the enzymes and facilitating the transfer of solids to the liquid phase. Therefore, Cellulase was included in the enzyme complex for all subsequent hydrolysis.

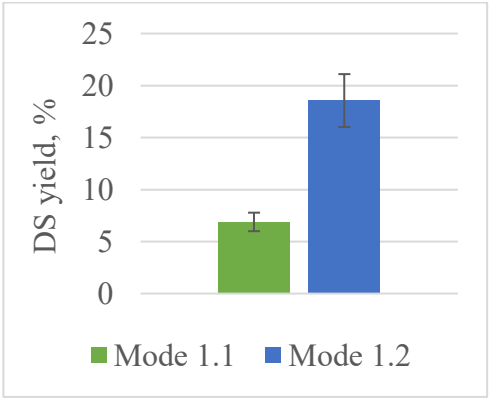


Figure 2. DS yield during hydrolysis with (Mode 1.2) and without (Mode 1.1) Cellulase.

The next step in improving the efficiency of enzymatic hydrolysis was the development of two modes using the same complex of enzyme preparations but differing in the timing of Cellulase addition and temperature conditions. The parameters of Mode 2.1 allowed all enzyme preparations to be added simultaneously at the beginning of the process, as the temperature and pH values were the most suitable for all, though not optimal for each. Mode 2.2, which included two temperature pauses, provided optimal working conditions for each enzyme.

According to the results (Figure 3), no plateau in DS concentration was observed in either mode, while the highest DS concentration at the beginning and end of

enzymatic hydrolysis was observed in Mode 2.2 (two temperature pauses).

For each mode, the DS yield rate was calculated hourly. According to the data (Figure 3), after four hours of enzymatic hydrolysis, there was a decrease in the DS yield rate. At the same time, Mode 2.2 was characterized by a higher rate, especially at the beginning of hydrolysis. The average DS yield rate during hydrolysis was 0.23 °Bx/h for Mode 2.1 and 0.26 °Bx/h for Mode 2.2.

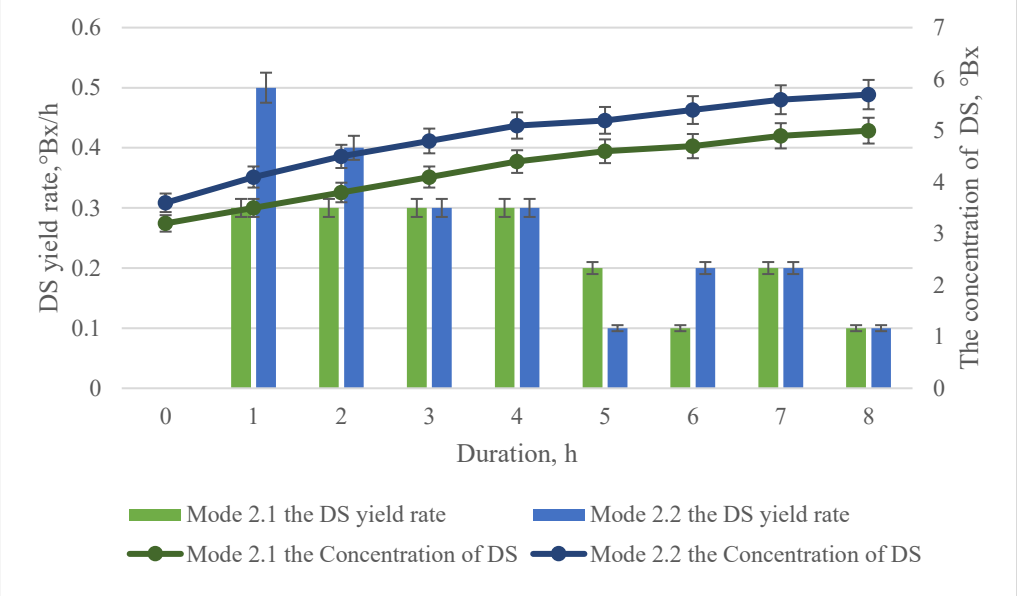


Figure 3. The DS yield rate and the concentration of DS change curves during hydrolysis in Modes 2.1 and 2.2.

It was also found that pressing COC hydrolysate with a hydraulic press increased the volume of the separated liquid fraction compared to separation by centrifugation from 33.87% to 70.25%. The necessity of pressing is probably due to the moisture-binding properties of COC. Thus, the extraction stage after enzymatic hydrolysis allows a larger volume of hydrolysate for subsequent use, for example, in yeast cultivation.

The effectiveness of the hydrolysis modes was evaluated based on DS yield values (Figure 4).

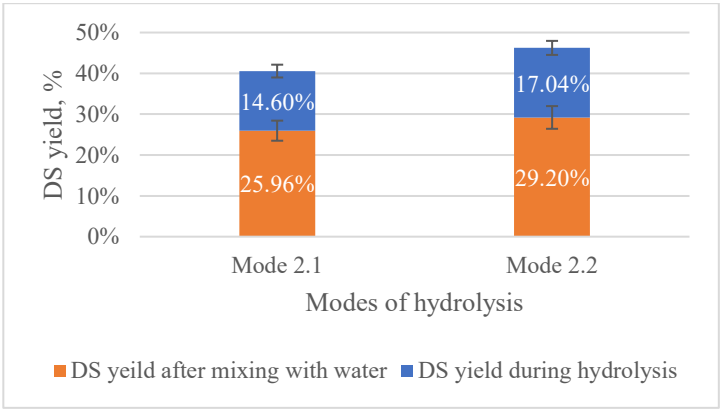


Figure 4. DS yield during hydrolysis in Modes 2.1 and 2.2.

Mode 2.2 produced the highest overall DS yield (46.24 ± 3.46%) and was selected as the most effective. It has been shown that during the hydrolysis itself, the DS yield of 17.04% was observed, while the remaining (29.20%) was extracted after mixing COC with water.

The separated COC hydrolysate liquid fraction had a DS concentration of 6.0 and a pH of 5.15. This concentration of DS enabled the hydrolysate to be used without additional dilution, thereby avoiding osmotic stress on the yeast.

The FAN concentration and the degree of substrate consumption in the COC hydrolysate were also determined and compared with those in malt wort, which served as a control (Table 4).

Table 4. Comparison of parameters of COC hydrolysate and malt wort (control).

Parameter	The FAN concentration, mg/100 ml	The substrate consumption degree, %
COC hydrolysate	40.20 ± 0.17	22.11 ± 0.53
Malt wort	10.31 ± 0.08	59.61 ± 1.06

The COC hydrolysate was characterized by a high FAN concentration, an important indicator of the nitrogen available for yeast assimilation in the medium, which could enhance yeast cultivation efficiency. However, the substrate consumption degree was almost three times lower than that of malt wort, which may be due to the unbalanced hydrolysate composition as a nutrient medium. This imbalance may lead to inhibition of yeast growth.

Cultivation of S. cerevisiae: The potential application of COC hydrolysate as a substrate for yeast cultivation was assessed using the growth coefficient and biomass yield.

The growth coefficient characterizes the increase in biomass during the cultivation compared to its initial value (Figure 5).

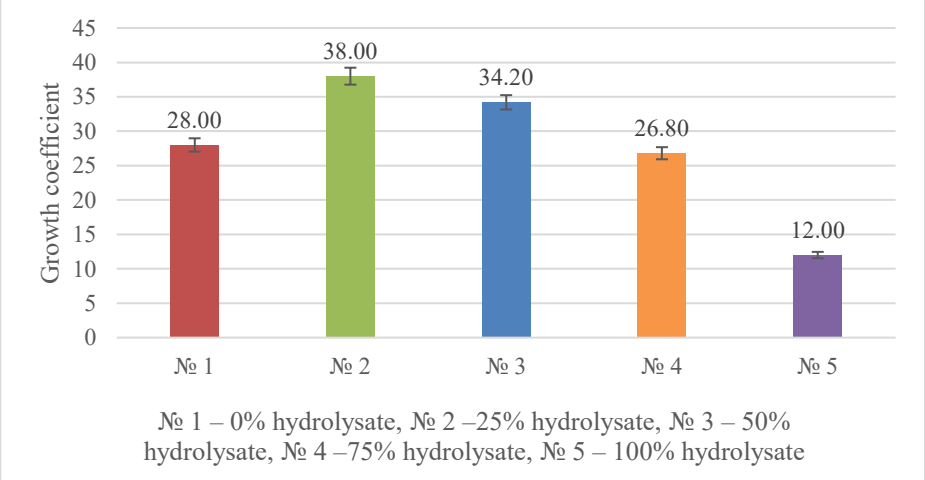


Figure 5. Growth coefficient during cultivation on nutrient media with different ratios of malt wort and COC hydrolysate.

The nutrient medium based on 100% COC hydrolysate is characterized by the lowest growth coefficient, equal to 12.00 ± 0.46. These values are more than twice as low as those observed during cultivation in malt wort. We observed the greatest increase in the growth coefficient, up to 38.00 ± 1.23, in the medium containing 25% COC hydrolysate, which can be attributed to a higher FAN concentration in the substrate. Simultaneously, as the proportion of COC hydrolysate in the composition increases, the concentration of sugars in the medium decreases, negatively affecting biomass accumulation and resulting in a lower growth coefficient.

Based on the cultivation results, the biomass yield was calculated (Figure 6), which determines the process efficiency relative to the carbohydrates consumed.

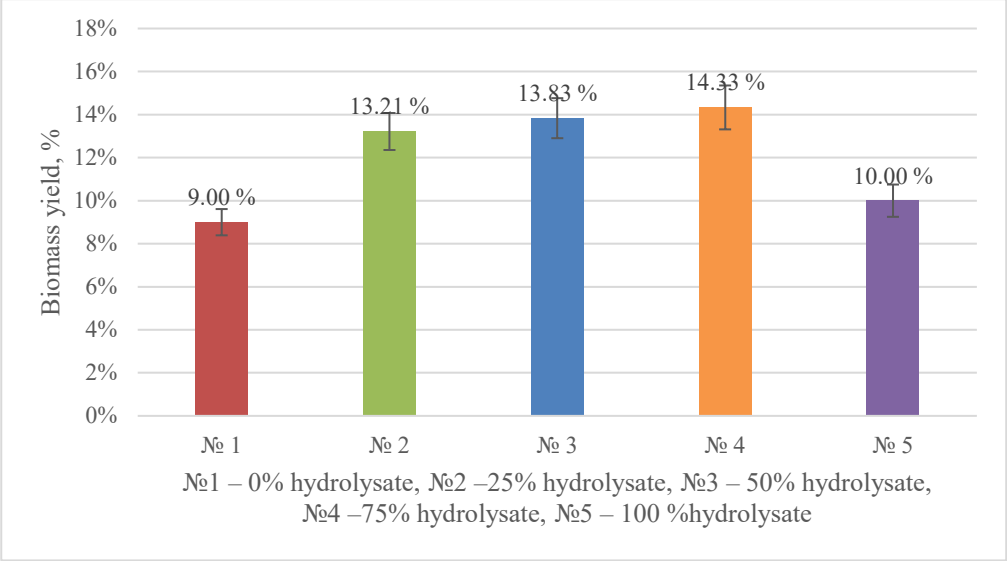


Figure 6. Biomass yield during cultivation on nutrient media with different ratios of malt wort and COC hydrolysate.

The biomass yield for cultivation using the 100% COC hydrolysate was 10.00 ± 0.75%, which is slightly higher than the value for malt wort (9.00 ± 0.61%). In the mixed media, biomass yield increased with increasing COC hydrolysate proportion.

Therefore, the most suitable medium for cultivation is one containing 25% COC hydrolysate, which shows the highest growth coefficient and a relatively high biomass yield (13.21 ± 0.86%) compared to the other media.

The results of this study can be used to develop functional food products with immunomodulatory and antioxidant effects due to the production of mannoproteins and β-glucans during *S. cerevisiae* cultivation on the proposed medium [24, 25]. The practical implication of the research is the potential to increase the production efficiency of bioactive compounds by reducing substrate costs using agricultural by-products.

CONCLUSION

The COC chemical composition is characterized by high protein, fat, and starch content. The most effective mode of COC enzymatic hydrolysis is a mode with two temperature pauses (75 ° C and 60 °C, each for four hours), which provides a DS yield of 46.24 ± 3.46%. The FAN concentration in the obtained hydrolysate is higher than in the malt wort. COC hydrolysate can be used as a substrate, but its most effective application is as an additive to malt wort at a proportion of 25%, which contributes to the increase in the growth coefficient and biomass yield during the *S. cerevisiae* cultivation.

Abbreviations: COC: corn oil cake, DM: dry matter, *S. cerevisiae*: *Saccharomyces cerevisiae*, DS: dissolved solids, FAN: free amine nitrogen, DB: dry biomass.

Competing Interests: There are no conflicts of interest to declare.

Author Contributions: V.N., T.M, and D.M. designed the research. V.N., E.Z., and D.M conducted the research. V.N. provided resources and supported the research. E.Z. and D.M. wrote the manuscript. E.Z. translated the manuscript. T.M and D.M. edited the manuscript. All authors read and approved the final version of the manuscript.

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