AGRICULTURAL BIOLOGY,
2021, V. 56, Iss. 6, pp. 1172-1182
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 664.872

doi: 10.15389/agrobiology.2021.6.1172eng
doi: 10.15389/agrobiology.2021.6.1172rus

A TECHNOLOGY FOR OBTAINING A PROTEIN CONCENTRATE FROM YEAST BIOMASS OF *Kluyveromyces marxianus* Van der Walt (1965)

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The authors declare no conflict of interests
Received July 8, 2021

Abstract

Lack of protein in the diet leads to a violation of nitrogen metabolism. Experts estimate that the feed protein market will exceed US $ 200 billion by 2024. In Russia, the deficit of fodder proteins is more than 1 million tons. Traditional protein sources cannot meet the daily need for food and feed proteins because of economic and social reasons, so there is a growing interest in alternative protein sources. This communication presents the results of obtaining a protein concentrate based on the biomass of thermotolerant yeast *K. marxianus* grown on a waste of oilseed production that is little used in bioconversion - sunflower husk. Yeast of this type increases the digestibility of feed, is used in the food industry for the fermentation of soy milk, soft cheese and as a flavor enhancer. The aim of the study was to develop a technology for obtaining a protein concentrate from the yeast biomass of *K. marxianus* and to substantiate the feasibility of its use as a feed and food additive. The *K. marxianus* Y-4570 strain was selected as a result of screening on sunflower husk fermentolysate as the most productive in terms of biomass accumulation (up to 30 g/l) and crude protein (59.29±2.96 %). Using a multicyclic semi-continuous method, yeast was cultured in a laboratory fermenter on a saline medium with sunflower husk fermentolysate to obtain protein biomass. Technological parameters were determined to obtain the protein concentrate containing at least 60 % of the true protein, no more than 2 % of lipids and no more than 2 % of nucleic acids. The biomass was defatted with 60 % ethyl alcohol with a hydromodule of 1:2.5 at 60 °C for 1 hour. The residual lipid content was 1.94±0.09 %. Denucleinization was performed by activating the cell's own endonucleases at 40-60 °C. Nucleic acids were removed at a 50 °C for 1 hour with a hydromodule 1:7. The residual content of nucleic acids was 1.97±0.10 %. The final product contains 65.94±3.14 % of true protein, which meets the requirements for protein concentrates. Analysis of the amino acid profile of the protein concentrate showed that the content of almost all essential amino acids exceeds that in the original yeast biomass, with the exception of glycine, leucine and histidine. A relative increase in the content of amino acids occurs due to the removed lipids, nucleic acids, the loss of moisture and the concentration of substances of the original biomass with drying. Protein concentrate based on the biomass of the yeast *K. marxianus* Y-4570 is intended for use as a feed and food additive in order to enrich products with essential amino acids.

Keywords: protein concentrate, protein, lipids, nucleic acids, *Kluyveromyces marxianus*, yeast, denuclearization, degreasing

In intensive animal farming, because of restrictions on the use of feed antibiotics, protein preparations are recently attracting increasing attention to improve animal health and accelerate the growth [1]. *Basidiomycetes* can be a source of complete protein. However, fungi grown in natural conditions are capable of accumulating toxic heavy metals [2, 3], therefore, under industrial conditions, champignons and oyster mushrooms are most often grown on artificial soils. Mushrooms are only 16.47-36.96% protein, which is their main disadvantage [4], although this figure for edible mushrooms such as champignons and oyster mushrooms is approximately 2 times higher than for vegetable crops [5]. Algae are rich in protein (on average up to 60-65% of dry matter) [6] and contain biolactive

substances that have a beneficial effect on humans and animal health (vitamins, minerals, antioxidants) [7]. However, algae, like champignons and oyster mushrooms, are able to accumulate heavy metals from the environment, so algae cannot be considered as a proper replacement for traditional protein sources either [8]. Among protein-oil crops, soybean is the leader in yield. However, discussions about the dangers of soybeans for human do not stop: its use is associated with the occurrence of cancer and the appearance of allergic reactions [9, 10].

The range of yeast feed preparations is quite wide. Dietary yeast primarily enrich feed with essential amino acids, in particular lysine [1, 11]. Yeast feed additives may be obtained from potato processing waste [12] and from the fermentation of cheese whey with the yeast *Kluyveromyces fragilis* [13]. Some yeasts have therapeutic and prophylactic effects on humans and animals [14]. Adding *Saccharomyces cerevisiae* protein preparations into feed increased the number of villi in the intestine and stimulated animal productivity [15-18]. The use of *Kluyveromyces marxianus* as a feed additive in fish farming provides a 40% replacement of the protein in expensive fishmeal without loss in salmon growth rates [19]. Yeast is a promising biologically active feed and food additive [20]. They accumulate up to 60% protein in dry mass, contain B vitamins [21, 22], some species, in particular *Saccharomyces cerevisiae*, serve as a rich source of ergosterol [23].

In microbial synthesis for protein production, cheap substrates [24, 25], in particular, cheese whey [13] and lignocellulose-containing waste and products of processing the cellulose-containing raw materials [26] are the indisputable advantages. Growing yeast biomass on chicken manure allows utilization of poultry waste that is toxic to the environment [27]. Microbiological bioconversion of agricultural waste into protein products reduces the negative impact on the environment [28].

Along with biologically valuable protein and vitamins, yeasts synthesize organic acids, polyhydric alcohols, and enzymes [29]. However, it is necessary to control the content of lipids and nucleic acids in yeast preparations [3]. Lipids cause unpleasant taste, odor, as they enter into oxidation reactions, and nucleic acids contain nitrogen, which accumulates in the form of urates, causing urolithiasis [30].

Kluyveromyces marxianus is an ascomycete yeast with pronounced thermotolerant properties used in the biotechnological production of enzymes, in particular inulinase, β-galactosidase and pectinase [31]. *K. marxianus* is also used in agriculture and the food industry, including for the production of ethanol, aromatics, and as starter cultures [32-35]. A number of data confirm the safety of the *Kluyveromyces* yeast for human and animal health [36, 37].

The main reasons hindering the industrial production of microbial protein using yeast fungi are the high cost of technologies due to expensive equipment and significant energy consumption during fermentation. For baker yeast *Saccharomyces cerevisiae* and *Candida*, *Cryptococcus*, and *Torulopsis* yeasts used for microbiological synthesis, the optimum growth temperature is 28-32 °C, while in *K. marxianus* it is 34-40 °C [1]. Yeast cultures are heat generating. Therefore, it is profitable to cool the medium in the bioreactor to a higher temperature, reducing the coolant consumption. In addition, from the point of view of waste bioconversion and cost reduction, it is important to expand the raw material base of such production. This report presents the results of obtaining a protein concentrate based on the biomass of thermotolerant yeast *K. marxianus* grown on sunflower husks, which are little used in bioconversion. The strain selected during screening on a nutrient medium containing enzymatic lysate of sunflower husk accumulates 59.29±2.96% of crude protein, the biomass yield reaches 30 g/l.

The study aimed to develop a technology for production of a protein concentrate from the *K. marxianus* yeast biomass and to substantiate the feasibility of its use as a feed and food additive.

Materials and methods. Sunflower husks were ground to a particle size of 30-100 μm (a rotor beater mill Retsch SR 200, RETSCH GmbH, Germany) controlled with a particle analyzer HELOS (H3908) & RODOS/L, R5 (Sympatec GmbH, Germany). The crushed particles were delignified by suspending in a 4% NaOH solution at a hydromodulus of 1:8.5 followed by incubation at 125±1 °C for 2 h; the extractant was separated by centrifugation. The resulting wet sediment of delignified husks was suspended in water and subjected to enzymatic hydrolysis for 24 h at 50±1 °C, pH 5.0±0.1. We used the enzyme preparation RovabioMax AP (Adisseo France S.A.S., France); cellulolytic activity 1900 units CA/g as per GOST R 55293-2012, xylanase activity 23500 units XA/g as per GOST R 55302-2012, the dosage of the preparation was determined at cellulase activity of 35 ClA/g raw material). After 24 h, the suspension was centrifuged, and the enzyme lysate was used as a substrate.

The yeast biomass of the *Kluyveromyces marxianus* Y-4570 strain (obtained from the collection of the NBC VKPM NRC Kurchatov Institute—GosNIIgenetika, Moscow) was produced using deep culture in a medium containing 0.50% $NH_4H_2PO_4$, 0.10% $MgSO_4$, 0.06% KH_2PO_4, 0.20% yeast extract, and up to 100% sunflower husk enzymatic lysate (8% DM).

Yeast was cultured in a multicyclic semi-continuous way in a laboratory fermenter MD-300 (L.E. MARUBISHI, Japan), aeration 1 v/(v · min) (air volume to nutrient medium volume), 38±1 °C, pH 5.0 (25% aqueous ammonia solution served as a titrant; alkalization of the medium indicated the need to add fresh enzyme lysate). A yeast suspension (4%) obtained in flasks on a liquid Sabouraud medium (5×10^6 cells/ml, counted with a Goryaev chamber) was used as an inoculum. After 10-day culture, the yeast biomass was separated at 5000 rpm for 15 min in a laboratory centrifuge (MLWT23D, OOO Medtehnika-Servis, Ukraine).

The amount of dry matter in the samples were determined gravimetrically by drying samples to constant weight at 105°°C. Crude protein in the biomass was measured according to Kjeldahl [38] (an automatic LK-500 distillation system. ZAO Laboratory Equipment and Devices, Russia). The analyzed sample was mineralized, ammonia was distilled off for 10 min in a Parnas-Wagner apparatus (PJSC Khimlaborpribor, Russia). The excess acid from the receiving flask was titrated with 0.1 N sodium hydroxide solution. The true protein was measured by the Barnstein method. The precipitate was filtered, washed, and the amount of nitrogen was determined by the Kjeldahl method [48]. Lipids were determined according to Folch [39] by distillation in a device for distilling liquids (NPO Laborkomplekt, Russia), and then drying to constant weight in a ShS-80-01 SPU drying cabinet (OAO Smolensk SKTB SPU, Russia). Nucleic acids were measured by Spirin method (an SSP-715 spectrophotometer, ZAO Spectroscopic Systems, Russia) [40].

The amino acid composition was analyzed by capillary electrophoresis (Kapel-105M system, Lumex LLC, Russia; the M-04-38-2009 methodology amended No. 1 of February 1, 2010, in accordance with the manufacturer's recommendations).

For defatting, the yeast biomass was extracted with ethyl alcohol (food alcohol Lux from grain raw materials; 40, 60 and 70%) in the ratio of biomass:ethanol 1:1.5; 1:2.0, and 1:2.5 in three doses of 20 min each. An appropriate amount of alcohol was added successively every 20 min, for a total of 60 min, at 50, 60 and 70 °C (a water bath TW-2.02, Elmi, Latvia). The partially defatted biomass was dried in an oven to constant weight. The amount of extracted lipids was

determined by the difference between the initial dry yeast biomass and the defatted biomass.

Nucleic acid degradation in yeast biomass occurred due to own enzyme activation at 50-70 °C. The incubation in a water bath took from 30 min to 1.5 h with hydromodules of 1:3, 1:5 and 1:7.

Statistical processing of quantitative data was performed using the STATISTICA 23.0 software package (StatSoft, Inc., USA). All measurements were performed in 3 replicates. Results are presented as weighted arithmetic mean (*WAM*) with standard deviation (±SD). Statistical significance was calculated using the non-parametric Mann-Whitney U-test and Kruskal-Wallis H-test. The critical significance level of the null hypothesis (p) was equal to 0.05.

Results. In the sunflower husk enzymatic lysate used to grow *K. marxianus* Y-4570 was 7.0-8.0% dry matter, 3.0-3.5% reducing substances (according to Bertrand), 69.65±3.48% glucose, 16.08±0.80% cellobioses, and 14.27±0.71% higher sugars.

The resulting *K. marxianus* Y-457 biomass was 59.29±2.96% crude protein, 13.45±0.67% lipids, and 8.85±0.44% nucleic acids. The residual amount of lipids and nucleic acids in protein preparations, which worsens their safety and provokes the formation of stones, should not exceed 2.00% [30].

The fundamental difference between our method and similar methods is the use of edible ethyl alcohol (95%) to remove lipids. For this purpose, the Folch method is most commonly used, extracting lipids with a mixture of chloroform:methanol (2:1 v/v) [41]. For food protein, it is advisable to use ethyl alcohol without chloroform. The amount of extracted lipids did not differ much compared to the Bligh-Dyer method, where chloroform is used for extraction in addition to ethyl alcohol, 33.04±0.16% without chloroform vs. 33.18±0.24% with chloroform according to the Bligh-Dyer method [42].

With an increase in the concentration of ethanol, the amount of extracted lipids increased, reaching a maximum for 70% ethanol (Table 1). However, it is preferable to use 60% ethanol, since 40% ethanol insufficiently removed lipids while the use of 70% alcohol increases costs, despite the fact that the amount of extracted lipids differs only by 1-2%. Ethanol 60% (1:2.5 v/v) removes up to 80% of lipids. In addition, sonication pre-treatment ensures the removal of a large amount of lipids [43].

1. Lipids (of initial content, %) **extracted from yeast *Kluyveromyces marxianus* biomass under various conditions** (n = 27, *WAM*±SD, lab test)

Temperature and ethanol concentration	Hydromodul		
	1:1.5	1:2.0	1:1.5
50 °C:			
40 %	72.41 ± 3.62^{Aa}	74.18 ± 3.70^{Ab}	75.02 ± 3.75^{Ac}
60 %	74.24 ± 3.71^{Bb}	75.24 ± 3.76^{Bc}	77.70 ± 3.88^{Bd}
70 %	75.12 ± 3.75^{Cc}	76.30 ± 3.81^{Cd}	78.52 ± 3.92^{Cf}
60 °C:			
40 %	75.46 ± 3.77^{Ca}	76.74 ± 3.83^{Cb}	82.90 ± 4.14^{Dd}
60 %	76.18 ± 3.80^{Cb}	78.10 ± 3.90^{Dc}	85.57 ± 4.27^{Ee}
70 %	77.12 ± 3.85^{Dc}	81.32 ± 4.06^{Ed}	86.46 ± 4.32^{Ee}
70 °C:			
40 %	78.54 ± 3.92^{Da}	82.15 ± 4.10^{Ec}	87.17 ± 4.35^{Fe}
60 %	79.60 ± 3.90^{Db}	83.47 ± 4.17^{Fd}	87.89 ± 4.39^{Fe}
70 %	81.04 ± 4.05^{Ec}	84.30 ± 4.21^{Fd}	89.55 ± 4.47^{Gf}

$^{A-G}$ Differences between the values in the column are statistically significant and differ at $p < 0.05$.
$^{a-f}$ Differences between the columns are statistically significant and differ at $p < 0.05$.

As the temperature increased, the amount of extracted lipids increased (see Table 1). The largest amount of lipids was remove at 70 °C (but this temperature regime is economically unfavorable), the smallest amount at 50 °C, therefore, a

temperature of 60 °C was chosen.

After choosing the concentration of ethanol and temperature, the ratio of ethanol:biomass was determined to extract the largest amount of lipids. Ethyl alcohol was used at ratios to biomass of 1:1.5; 1:2.0 and 1:2.5. With 60% ethanol and 60 °C, the optimal ethanol:biomass ratio was 1:2.5 (see Table 1), resulting in 85.57±4.27% extracted lipids. For 1:1.5 and 1:2.0 ratios, less lipids were removed, and their residual amount was more than 2.0%. In a similar study, the following parameters were proposed, the biomass:ethanol ratio 1:40, 135 °C and P = 1.5 MPa [44]. In this case, the disadvantages are the high consumption of the extractant, the high temperature which negatively affects the amino acid composition of the protein concentrate, and the use of excess pressure which leads to additional costs.

Thus, based on the data obtained, we propose the ratio of yeast biomass:60% ethanol of 1:2.5 at a temperature of 60 °C as technological parameters for lipid extraction. These treatments reduce the residual lipid content to 1.94±0.09%. One of the works reported on the preparation of a baker's yeast protein concentrate in which the amount of residual lipids after extraction was 6.47% [45].

The extraction temperature has the greatest influence on the amount of extracted nucleic acids (factor load 0.700).

With yeast biomass:water ratio of 1:7, the samples were kept at 40, 50 and 60 °C in a water bath for 0.5; 1 and 1.5 h for nucleic acid degradation. Less amount of nucleic acids was removed at 40 °C compared to 50 and 60 °C (see Table 2). It can be concluded that with an increase in temperature to a certain level, the activity of the yeast's own enzymes will increase, i.e., at 40 °C it is lower than at 50 °C) while at 60 °C the enzymes inactivation occurs. At 70 °C, nucleic acid residual was approximately 3.00%, but the amount of lysine also reduced. At 50 °C, nucleic acids would account for approximately 2.50%, while lysine loss would not be so significant [46].

2. Nucleic acids (of initial content, %) **extracted from yeast *Kluyveromyces marxianus* biomass under various conditions** (n = 27, *WAM*±SD, lab test)

Temperature and extraction time	Hydromodul		
	1:3	1:5	1:3
40 °C:			
0.5 h	56,32±2,81^{Aa}	58,75±2,93^{Ab}	59,60±2,98^{Ab}
1.0 h	58,60±2,93^{Bb}	60,12±3,00^{Bd}	64,02±3,20^{Be}
1.5 h	62,75±3,13^{Cc}	65,43±3,27^{Ce}	66,05±3,30^{Ce}
50 °C:			
0.5 h	67,89±3,39^{Da}	68,98±3,44^{Db}	70,25±3,51^{Dc}
1.0 h	69,34±3,46^{Eb}	71,30±3,56^{Ed}	77,74±3,88^{Ee}
1.5 h	70,02±3,50^{Ec}	72,46±3,62^{Ed}	79,88±3,99^{Ef}
60 °C:			
0.5 h	66,16±3,30^{Da}	67,18±3,35^{Db}	68,07±3,40^{Cc}
1.0 h	67,25±3,36^{Db}	68,93±3,44^{Dc}	69,74±3,48^{Dd}
1.5 h	68,73±3,43^{Ec}	69,80±3,49^{Dd}	70,10±3,50^{Df}

$^{A-D}$ Differences between the values in the column are statistically significant and differ at $p < 0.05$.
$^{a-f}$ Differences between the columns are statistically significant and differ at $p < 0.05$.

The final step was to determine the optimal time for nucleic acid degradation. In our experiment, it was 1.0 h (see Table 2). At 50 °C for 1 h and hydromodule of 1:7, up to 77.74±3.88% nucleic acids were removed. Thus, these parameters were selected for nucleic acid degradation.

Pacheco et al. [47] developed a technology for the production of a protein concentrate from baker's yeast with a true protein content of about 75.0% on average, for which salts (sodium perchlorate and sodium trimetaphosphate) were used. In this regard, we note that there are reports of a negative effect of sodium perchlorate on thyroid function [48]. In our work, ethyl alcohol and native yeast endonucleases were used to obtain the concentrate. In a similar study,

the biochemical composition of the *K. marxianus* and *S. cerevisiae* autolysates was compared [49]. The *K. marxianus* biomass contains a large amount of nucleic acids (approximately 10%) and 56% of true protein vs. approximately 9% and 57% for *S. cerevisiae* [30]. In our work (Table 3), the *K. marxianus* initial biomass was 13.45±0.67% lipids and 8.85±0.44% nucleic acids vs. 1.94±0.09% and 1.97±0.10%, respectively, for the produced protein concentrate.

3. Biochemical composition (of dry matter, %) **of initial yeast *Kluyveromyces marxianus* biomass and the protein concentrate after extraction of lipids and nucleic acids** (n = 8, *WAM*±SD, lab test)

Parameter	Biomass	Protein concentrate
Crude protein	59.29 ± 2.96^{Aa}	71.65 ± 3.43^{Bb}
True protein	54.60 ± 2.73^{Aa}	65.94 ± 3.14^{Cc}
Lipids	13.45 ± 0.67^{Da}	1.94 ± 0.09^{Fd}
Nucleic acids	8.85 ± 0.44^{Ea}	1.97 ± 0.10^{Fd}

$^{A\text{-}F}$ Differences between the values in the column are statistically significant and differ at $p < 0.05$.
$^{a\text{-}d}$ Differences between the columns are statistically significant and differ at $p < 0.05$.

The yeast biomass protein of *K. marxianus* was lower in the contents of lysine, threonine, and sulfur-containing amino acids (Table 4). However, the resulting protein concentrate was almost completely balanced in all essential amino acids, except for sulfur-containing amino acids, phenylalanine and tyrosine. Processing under selected technological parameters significantly increased the content of lysine, threonine, serine, arginine, proline, glutamine and aspartic amino acids, however, as compared to the initial biomass, the amount of leucine, histidine and glycine decreased. The relative increase in the content of amino acids occurred due to a decrease in the content of lipids, nucleic acids and the removal of moisture during drying. Compared to the initial biomass, the lysine level increased by 1.75%, threonine by 0.50%, serine by 0.62%, arginine by 1.02%, proline by 2.66%, aspartic acid by 1.93%, and glutamic acid by 1.76%. The amount of glycine decreased by 2.14%, leucine by 1.22%, and histidine by 0.16%, since heating destroys these amino acids.

4. Amino acid composition (g/100 g protein) **of initial yeast *Kluyveromyces marxianus* biomass and the protein concentrate after extraction of lipids and nucleic acids as compared to the FAO ideal protein** (*WAM*±SD, lab test)

Amino acid	Yeast biomass	Protein concentrate	"Ideal" protein
Phenylalanine + tyrosine	5.77±0.28	5.20±0.26	6.0
Leucine	6.70±0.33	5.48±0.27	5.9
Lysine	3.75±0.18	5.50±0.27	5.5
Valine	4.36±0.21	4.86±0.24	4.9
Isoleucine	4.28±0.22	4.35±0.21	4.0
Threonine	1.87±0.09	2.31±0.11	3.3
Tryptophan	1.23±0.06	1.34±0.06	1.0
Glutamic acid	2.55±0.12	4.31±0.21	–
Arginine	3.46±0.17	4.48±0.22	–
Glycine	4.59±0.23	2.45±0.12	–
Aspartic acid	3.54±0.17	5.47±0.27	–
Methionine + cysteine	2.03±0.10	2.20±0.11	3.5
Proline	2.61±0.13	5.27±0.26	–
Histidine	1.82±0.09	1.66±0.08	1.5
Alanine	4.32±0.21	5.53±0.27	–
Serine	0.41±0.02	1.03±0.05	–

N o t e. Dashes mean that these amino acids are not determined for the "ideal" protein.

Lysine is the main limiting amino acid in pig feed. In the protein concentrate we obtained its amount is 5.50±0.27%. This is enough to meet the daily needs of farm animals. For birds, the limiting amino acids are cysteine and methionine the amount of which in the protein concentrate (2.2±0.11%) can also cover daily requirement. One study used a yeast protein concentrate to feed *Cyprinidae* fish [50] and showed that this concentrate could replace up to 50% of

expensive fishmeal in carp diets without any negative effects on fish health and growth. In addition, the amino acid composition of the yeast biomass of *K. marxianus* and the protein concentrate is comparable in composition to the ideal FAO/WHO protein [51, 52] (see Table 4).

The main advantage of our technology is the use of thermotolerant yeast *K. marxianus*, which can be cultured at higher temperatures (34-40 °C). In addition, to date, most studies have focused on the physiology and metabolism rather than on practical applications of *K. marxianus* [32]. Baker's yeast is most commonly culture at approximately 32 °C [53]. Yeast culture generates a large amount of heat, so it is necessary to cool the medium in the bioreactor. *K. marxianus* yeast requires less cooling than baker's yeast, which reduces the amount of coolant.

Using the developed technology, we have obtained a prototype protein concentrate. It is a paste-like mass after drying in an oven at 103 °C to a 5.56% residual moisture content. Characteristics of the prototype are as follows:

Parameter	Regulatory document	Показатель
Crude protein	GOST 20083-74	71.65 %
True protein	GOST 20083-74	65.94 %
Appearance	GOST P 54731-2011	Homogeneous dry fine powder
Color	GOST P 54731-2011	Light beige or light brown
Flavor	GOST P 54731-2011	Yeast-specific, without off-flavours
Taste	GOST P 54731-2011	Yeast-specific, without extraneous aftertaste
Moisture	GOST P 54731-2011	5.56 %
Microbiological indicators	Technical regulation of the Customs Union 021/2011	Matched
Heavy metal content	Technical regulation of the Customs Union 021/2011	Matched

Thus, the sunflower oil production generates a large amount of wastes. We have developed the biotechnology for manufacturing a protein-rich product from the yeast *Kluyveromyces marxianus* Y-4570 biomass with the use of sunflower husk enzymatic lysate as the culture medium. The technology includes nucleic acid degradation and defattening. Lipid removal includes extraction with 60% ethanol for 1.0 h at 60 °C and a hydromodule of 1:2.5 (the residual amount of lipids does not exceed 2%). Nucleic acid degradation occurs during 1.0 h due to yeast's own endonucleases at 50 °C and a hydromodulus of 1:7 (the residual amount of nucleic acids also does not exceed 2%). The resultant protein concentrate contains at least 65% of true protein. The increase in the content of amino acids in the protein concentrate occurs due to a decrease in the content of lipids and nucleic acids. The amino acid composition of yeast biomass and protein concentrate is comparable to that of an ideal FAO/WHO protein. The introduction of a concentrate from the yeast biomass of *K. marxianus* Y-4570 into food products will enrich them with protein with a high content of essential amino acids and improve the organoleptic qualities. Protein concentrate can also be used in animal husbandry.

REFERENCES

1. Shurson G.C. Yeast and yeast derivatives in feed additives and ingredients: Sources, characteristics, animal responses, and quantification methods. *Animal Feed Science and Technology*, 2018, 235: 60-76 (doi: 10.1016/j.anifeedsci.2017.11.010).
2. Cocchi L., Vescovi L., Petrini L.E., Petrini O. Heavy metals in edible mushrooms in Italy. *Food Chemistry*, 2006, 98(2): 277-284 (doi: 10.1016/j.foodchem.2005.05.068).
3. Demirba A. Accumulation of heavy metals in some edible mushrooms from Turkey. *Food Chemistry*, 2000, 68(4): 415−-419 (doi: 10.5772/52771).
4. Bach F., Helm C.V., Bellettini M.B., Maciel G.M., Haminiuk C.W.I. Edible mushrooms: a potential source of essential amino acids, glucans and minerals. *International Journal of Food Science & Technology*, 2017, 52(11): 2382-2392 (doi: 10.1111/ijfs.13522).
5. Hamzah R.U., Jigam A.A., Makun H.A., Egwim E.C. Antioxidant properties of selected African vegetables, fruits and mushrooms: a review. In: *Mycotoxin and food safety in developing countries*. H. Makun. IntechOpen, London, 2013: 203-250 (doi: 10.5772/52771).
6. Becker E.W. Micro-algae as a source of protein. *Biotechnology Advances*, 2007, 25(2): 207-210

(doi: 10.1016/j.biotechadv.2006.11.002).
7. Wells M.L., Potin P., Craigie J.S., Raven J.A., Merchant S.S., Helliwell K.E., Brawley S.H. Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, 2017, 29(2): 949-982 (doi: 10.1007/s10811-016-0974).
8. Arulkumar A., Nigariga P., Paramasivam S., Rajaram R. Metals accumulation in edible marine algae collected from Thondi coast of Palk Bay, Southeastern India. *Chemosphere*, 2019, 221: 856-862 (doi: 10.1016/j.chemosphere.2019.01.007).
9. Fallon S., Enig M. Soy alert-tragedy and hype. *Nexus Magazine*, 2000, 7(3): 17-23.
10. Savage J.H., Kaeding A.J., Matsui E.C., Wood R.A. The natural history of soy allergy. *Journal of Allergy and Clinical Immunology*, 2010, 125(3): 683-686 (doi: 10.1016/j.jaci.2009.12.994).
11. Wang W., Li Z., Lv Z., Zhang B., Lv H., Guo Y. Effects of *Kluyveromyces marxianus* supplementation on immune responses, intestinal structure and microbiota in broiler chickens. *PLoS ONE*, 2017, 12(7): e0180884 (doi: 10.1371/journal.pone.0180884).
12. Gélinas P., Barrette J. Protein enrichment of potato processing waste through yeast fermentation. *Bioresource Technology,* 2007, 98(5): 1138-1143 (doi: 10.1016/j.biortech.2006.04.021).
13. Ghaly A.E., Kamal M.A. Submerged yeast fermentation of acid cheese whey for protein production and pollution potential reduction. *Water Research*, 2004, 38(3): 631-644 (doi: 10.1016/j.watres.2003.10.019).
14. Palma M.L., Zamith-Miranda D., Martins F.S., Bozza F.A., Nimrichter L., Montero-Lomeli M., Douradinha B. Probiotic Saccharomyces cerevisiae strains as biotherapeutic tools: is there room for improvement. *Applied Microbiology and Biotechnology*, 2015, 99(16): 6563-6570 (doi: 10.1007/s00253-015-6776-x).
15. Haldar S., Ghosh T.K., Bedford M.R. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis*. *Animal Feed Science and Technology,* 2011, 168(1-2): 61-71 (doi: 10.1016/j.anifeedsci.2011.03.007).
16. Moallem U., Lehrer H., Livshitz L., Zachut M., Yakoby S. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. *Journal of Dairy Science*, 2009, 92(1): 343-351 (doi: 10.3168/jds.2007-0839).
17. Liu S., Shah A.M., Yuan M., Kang K., Wang Z., Wang L., Xue B., Zou H., Zhang X., Yu P., Wang H., Tian G., Peng Q. Effects of dry yeast supplementation on growth performance, rumen fermentation characteristics, slaughter performance and microbial communities in beef cattle. *Animal Biotechnology*, 2021: 1-11 (doi: 10.1080/10495398.2021.1878204). Epub ahead of print.
18. Mogensen G., Salminen S., Crittenden R., Bianchi Salvadori B., Zink R. Inventory of microorganisms with a documented history of use in food. *Bulletin-International Dairy Federation*, 2002, 377: 10-19 (doi: 10.1016/j.ijfoodmicro.2011.12.030).
19. Øverland M., Karlsson A., Mydland L.T., Romarheim O.H., Skrede A. Evaluation of *Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, 2013, 402: 1-7 (doi: 10.1016/j.aquaculture.2013.03.016).
20. Rakowska R., Sadowska A., Dybkowska E., Swiderski F. Spent yeast as natural source of functional food additives. *Roczniki Państwowego Zakładu Higieny*, 2017, 68(2): 115-121.
21. Pérez-Torrado R., Gamero E., Gymez-Pastor R., Garre E., Aranda A., Matallana E. Yeast biomass, an optimised product with myriad applications in the food industry. *Trends in Food Science & Technology*, 2015, 46(2): 167-175 (doi: 10.1016/j.tifs.2015.10.008).
22. Ferreira I., Pinho O., Vieira E., Tavarela J.G. Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. *Trends in Food Science & Technology*, 2010, 21(2): 77-84 (doi: 10.1016/j.tifs.2009.10.008).
23. Hu Z., He B., Ma L., Sun Y., Niu Y., Zeng B. Recent advances in ergosterol biosynthesis and regulation mechanisms in Saccharomyces cerevisiae. *Indian Journal of Microbiology*, 2017, 57(3): 270-277 (doi: 10.1007/s12088-017-0657-1).
24. Gervasi T., Pellizzeri V., Calabrese G., Di Bella G., Cicero N., Dugo G. Production of single cell protein (SCP) from food and agricultural waste by using *Saccharomyces cerevisiae*. *Natural Product Research*, 2018, 32(6): 648-653 (doi: 10.1080/14786419.2017.1332617).
25. Jin Y.S., Cate J.H. Metabolic engineering of yeast for lignocellulosic biofuel production. *Current Opinion in Chemical Biology*, 2017, 41: 99-106 (doi: 10.1016/j.cbpa.2017.10.025).
26. Kistaubayeva A., Savitskaya I., Shokataeva D., Zhabakova A., Kuli Z. Utilization of agricultural waste by yeast-bacterial conversion of cellulose-containing substrates to protein feed products. *Eurasian Journal of Ecology*, 2017, 51(2): 34-43 (doi: 10.26577/EJE-2017-2-765).
27. Yan Z., Liu X., Yuan Y., Liao Y., Li X. Deodorization study of the swine manure with two yeast strains. *Biotechnology and Bioprocess Engineering*, 2013, 18(1): 135-143 (doi: 10.1007/s12257-012-0313-x).
28. Matassa S., Boon N., Pikaar I., Verstraete W. Microbial protein: future sustainable food supply route with low environmental footprint. *Microbial Biotechnology*, 2016, 9(5): 568-575 (doi: 10.1111/1751-7915.12369).
29. Halász A., Lásztity R. *Use of yeast biomass in food production*. CRC Press, 1991 (doi: 10.1201/9780203734551).
30. Ivanova I.S. *Razrabotka tekhnologii biologicheski aktivnoi dobavki k pishche v vide belkovo-*

uglevodnogo kontsentrata iz biomassy khlebopekarnykh drozhzhei. Kandidatskaya dissertatsiya [Development of technology for bioactive food additive in the form of protein-carbohydrate concentrate from baker's yeast biomass. PhD Thesis]. Moscow, 2003 (in Russ.).
31. Lane M.M., Burke N., Karreman R., Wolfe K.H., O'Byrne C.P., Morrissey J.P. Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*. *Antonie Van Leeuwenhoek*, 2011, 100(4): 507-519 (doi: 10.1007/s10482-011-9606-x).
32. Fonseca G.G., Heinzle E., Wittmann C., Gombert A.K. The yeast *Kluyveromyces marxianus* and its biotechnological potential. *Applied Microbiology and Biotechnology*, 2008, 79(3): 339-354 (doi: 10.1007/s00253-008-1458-6).
33. Karim A., Gerliani N., Aıder M. *Kluyveromyces marxianus*: an emerging yeast cell factory or applications in food and biotechnology. *International Journal of Food Microbiology*, 2020, 333: 108818 (doi: 10.1016/j.ijfoodmicro.2020.108818).
34. Varela J.A., Gethins L., Stanton C., Ross P., Morrissey J.P. Applications of *Kluyveromyces marxianus* in biotechnology. In: *Yeast diversity in human welfar*e. Springer, Singapore, 2017: 439-453 (doi: 10.1007/978-981-10-2621-8_17).
35. Lee M.H., Lin J.J., Lin Y.J., Chang J.J., Ke H.M., Fan W.L., Li W.H. Genome-wide prediction of CRISPR/Cas9 targets in *Kluyveromyces marxianus* and its application to obtain a stable haploid strain. *Scientific Reports*, 2018, 8(1): 1-10 (doi: 10.1038/s41598-018-25366-z).
36. Fadda M.E., Mossa V., Deplano M., Pisano M.B., Cosentino S. In vitro screening of *Kluyveromyces* strains isolated from Fiore Sardo cheese for potential use as probiotics. *Food Science and Technology*, 2017, 75: 100-106 (doi: 10.1016/j.lwt.2016.08.020).
37. EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). *EFSA Journal*, 2013, 11(11): 3449 (doi: 10.2903/j.efsa.2013.3449).
38. Weigand E., Kirchgessner M. Protein and energy value of vinasse for pigs. *Animal Feed Science and Technology*, 1980, 5(3): 221
39. Folch J., Lees M., Sloane-Stanley G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 1957, 226: 497-509 (doi: 10.1016/S0021-9258(18)64849-5).
40. Karklinya V.A., Birska I.A., Limarenko Y.A. Quantitative determination of nucleic acids in *Salmonidae* milt by various methods. *Chem. Nat. Compd.*, 25, 1989: 109-112 (doi: 10.1007/BF00596713).
41. Schneiter R., Daum G. Extraction of yeast lipids. *Methods Mol. Biol.*, 2006, 313: 41-45 (doi: 10.1385/1-59259-958-3:041).
42. Yang F., Xiang W., Sun X., Wu H., Li T., Long L. A novel lipid extraction method from wet microalga *Picochlorum* sp. at room temperature. *Marine Drugs*, 2014, 12(3): 1258-1270 (doi: 10.3390/md12031258).
43. Kot A.M., Gientka I., Bzducha-Wrybel A., Błażejak S., Kurcz A. Comparison of simple and rapid cell wall disruption methods for improving lipid extraction from yeast cells. *Journal of Microbiological Methods*, 2020, 176: 105999 (doi: 10.1016/j.mimet.2020.105999).
44. Chen M., Chen X., Liu T., Zhang W. Subcritical ethanol extraction of lipid from wet microalgae paste of *Nannochloropsis* sp. *Journal of Biobased Materials and Bioenergy*, 2011, 5(3): 385-389 (doi: 10.1166/jbmb.2011.1157).
45. Caballero–Córdoba G.M., Sgarbieri V.C. Nutritional and toxicological evaluation of yeast (*Saccharomyces cerevisiae*) biomass and a yeast protein concentrate. *Journal of the Science of Food and Agriculture*, 2000, 80(3): 341-351 (doi: 10.1002/10970010(200002)80:3%3C341::AIDJSFA533%3E3.0.CO;2-M).
46. Hedenskog G., Ebbinghaus L. Reduction of the nucleic acid content of single - cell protein concentrates. *Biotechnology and Bioengineering*, 1972, 14(3): 447-457 (doi: 10.1002/bit.260140313).
47. Pacheco M.T.B., Caballero-Cordoba G.M., Sgarbieri V.C. Composition and nutritive value of yeast biomass and yeast protein concentrates. *Journal of Nutritional Science and Vitaminology*, 1997, 43(6): 601-612 (doi: 10.3177/jnsv.43.601).
48. Leung A.M., Pearce E.N., Braverman L.E. Perchlorate, iodine and the thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism*, 2010, 24(1), 133-141 (doi: 10.1016/j.beem.2009.08.009).
49. Lukondeh T., Ashbolt N.J., Rogers P.L. Evaluation of *Kluyveromyces marxianus* as a source of yeast autolysates. *Journal of Industrial Microbiology and Biotechnology*, 2003, 30(1): 52-56 (doi: 10.1007/s10295-002-0008-y).
50. Omar S.S., Merrifield D.L., Kühlwein H., Williams P.E., Davies S.J. Biofuel derived yeast protein concentrate (YPC) as a novel feed ingredient in carp diets. *Aquaculture*, 2012, 330: 54-62 (doi: 10.1016/j.aquaculture.2011.12.004).
51. Andrade J., Pereira C.G., de Almeida J.C. Jr., Viana C.C.R., Neves de Oliveira L.N., Fonseca da Silva P.H., Bell, M.J.V., de Carvalho dos Anjos V. FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. *LWT*, 2019, 99: 166-172 (doi: 10.1016/j.lwt.2018.09.079).
52. Consultation F.E. *Dietary protein quality evaluation in human nutrition. FAO Food and Nutrition Paper, 92.* Rome, 2013.
53. Salvady Z., Arroyo-López F.N., Guillamyn J.M., Salazar G., Querol A., Barrio E. Temperature adaptation markedly determines evolution within the genus *Saccharomyces*. *Applied and Environmental Microbiology*, 2011, 77(7): 2292-2302 (doi: 10.1128/aem.01861-10).