Production of Protein-Rich Biomass by the Consortium of Microscopic Fungi - *Chaetomium cellulotycum* A 43 and *Sporotrichum pulverulentum* A 32

Izolda Khokhashvili[1] , Lali Kutateladze[1], Nino Zakariashvili [1], Tamar Urushadze[1],Maya Jobava[1] , Rusudan Khvedelidze[1,] Tamar Burduli[1]NinoTsiklauri[1] , Tinatin.Aleksidze[1].

[1] *Agricultural University of Georgia, S.Durmishidze Institute of Biochemistry and Biotechnology, University Campus of Digomi, David Agmashenebeli Alley 240, Tbilisi 0159, Georgia*.

Abstract **-** *Nonpathogenic and nontoxic strains of microscopic fungi, from the collection of the Institute of Biochemistry and Biotechnology of the Agricultural University of Georgia have been screened for the selection of proteins active producers under the conditions of solid phase fermentation of the agricultural and food industry waste. Two active producers of proteins have been revealed: C. cellulotycum A 43 and S. pulverulentum A 32, as well as the perspective substrate for the bioconvertion – tomato-cake was selected. The optimal conditions for cultivation and composition of the nutritional medium for Ch. cellulotycum A 43 and Sp.pulverulentum A32 were established. Biomasses with 2.3-2.8 times higher content of pure protein, compared with control have been obtained on the base of optimization of the cultivation conditions and nutritional medium composition. The united cultivation of experimental strains was performed for the improvement of bioconversion degree. The synergetic effect of the combined cultivation of fungi on the tomato-cake has been demonstrated. The combined cultivation of C. cellulotycum A 43 and S. pulverulentum A 32 under the optimal conditions resulted in the production of sugar- (20%) and protein-rich (16.5%), easy-digestible, nontoxic and nonpathogenic biomass, which may be used as protein- and other bioactive substances-rich food additive.*

Keywords: *Microscopic fungi; Solid state fermentation; Agricultural and food industry waste; Bioconversion; Protein-rich biomass;*

I. INTRODUCTION

According to existing data the annual deficiency of the high-nutritional value protein makes millions of tones in the world. Production of the enriched with functional ingredients food additives, on the base of the microbiological conversion of agricultural and food industry, is considered as the most convenient, ecologically significant role in such technologies. That's why revealing of the perfect producers, optimization its cultivation and safe and fast-realizable technology to fill the food deficiency [1], [2], [3]. Almost all waste of tinned-industry, post-harvesting residues of fruit and vagatables, crop stubble, vine,tea and orchard cuttings, etc., which is useless as food, occupies some territory and may even become the reason for local ecological misbalance, is a perspective substrate for biotransformation.

Traditionally yeast and bacteria are used for production of microbial protein. But microorganisms of these groups are able to use only easy-digestible substrates and are unable to degrade the lingo-cellulose waste [4],[5],[6]. Moreover, in the bacterial and yeast biomass content of nucleic acids is very high and additional processing is needed to decrease them; This procedure from its side increases the coast of the production [7].

The special group of micellar fungi – microscopic fungi reveal the high permeability of the mycelium into the plant substrates and low content of nucleic acids in the formed biomass; thanks to mighty hydrolytic enzymes they are able to assimilate hard-degradable polymers of plant waste and enrich the substrate with amino acids, sugars, polysaccharides, vitamins and other physiologically active compounds during the growth. [8], [9], [10]. This group of microorganisms is valuable by the taste quality as well.

By the content of particular amino acids fungal mycelium is close to soybean and animal protein; it is rich of cereal's deficient amino acid – lysine, that makes possible to produce balanced food additives by the mixing of cereals' and fungal biomasses [11].

Accordingly, the bioconversion of agro-industrial waste by the microscopic fungi may be considered as the real possibility of: food problem salvation, agricultural waste utilization, natural resources saving and environment protection. Certainly, the industrial strain plays a significant role in such technologies. That's why revealing nutritional

conditions and elaboration of the technological basics of food additives industry is very popular.

II. MATERIALS AND METHODS

A. cultivation of microscopic fungi

Nonpathogenic and nontoxic cultures of microscopic fungi, taken from the collection of the Institute of Biochemistry and Biotechnology of the Agricultural University of Georgia, served as test objects.

The submerged cultivation of microscopic fungi was performed for the purpose to study its cellulase activity. 50ml of nutritional medium (g/l): $NaNO_3$ - 3,0; KH_2PO_4 - 1.0; $MgSO_4 \cdot 7H_2O$- 0,5; $FeSO_4 \cdot 7H_2O$ - 0,02; microcrystalic cellulose – 0.5, corn extract – 1ml; pH=4.5, was placed in 250ml volume conic retorts and shaked (150 rot/min) at 32°C during 4 days.

B. Enzyme activities assay

Cellulase activity of the filter paper was determined according to Ghose method [12] 0, 5 ml enzyme, diluted in acetate buffer ,was added toWhatman No.1 filter paper strip (1×6cm; 50mg) immersed in one milliliter of 0.05M acetate buffer, pH 4,5 at 50 °C for 1h. The enzyme's activity was calculated by the amount of reducing sugars, which was released after the application of dinitrosalicylic acid reagent (DNS) [13].

To investigate the ligninase activity the microscopic fungi were cultivated on the same composition nutritional medium with one difference: glucose (1%) was used as a source of carbon and phenolic substance induline (10µmol/l) was added as well. Retorts were sterilized in autoclaves at 0.7atm, during 45min. At the end of cultivation the content of retorts was centrifuged at 4000rot/min for 10min. Ligninase activity was determined by the oxidizing of veratric alcohol [14]

C. Selection of the agro-industrial waste

agro-industrial waste: apple- and tomato-cakes, powder of citrus and orange peels, cereal's stubble (wheat and corn) and vine cuttings were applied in studies.

Content of the main components of the agro-industrial waste was determined.

Cellulose content in was determined according to Updegraff [15]. Hemicellulose was determined by treating the sample with 0.1N H_2SO_4. The amount of lignin was determined by treating the sample with 72% H_2SO_4. [16]. Biomass was treated with trichloracetic acid for the determination of pure protein. Percentage of the protein was calculated after [17]. Dry weight was determined by weight method according to difference between initial and final weights, by bringing sample to constant weight at 105˚C.

D. Solid phase fermentation of agro-industrial waste

The solid phase fermentation of agro-industrial waste: apple- and tomato-cakes, powder of citrus and orange peels, cereal's stubble (wheat and corn) and vine cuttings - was performed in thermostat at 32°C, stationary, during 8days. For this purpose 4g of absolutely dry, finely milled (0.4-0.5mm size particles) plant waste material was placed in 100ml volume conic retorts and added with 12ml of nutritional medium (g/l): $NaNO_3$ - 3,0; KH_2PO_4 - 1.0; $MgSO_4 \cdot 7H_2O$ - 0,5; $FeSO_4 \cdot 7H_2O$-0,02. pH of the nutritional medium was bring to 5.5-6.0 by 5% solution of NaOH. Retorts were sterilized in autoclave, under one atm. for 45min., cooled and added with 2ml of fungal spore suspension.

After the cultivation was finished, the content of the retort was transmitted by means of spatula into the constant mass vessel and placed into the thermostat at 105°C to be dried and brought to the constant weight. Differences between the initial and final masses of the sample corresponded to the biomass, which stayed after the bioconversion

E. Optimization of the composition of nutritional medium and cultivation conditions

Optimization of the composition of nutritional medium and cultivation conditions was performed by the standard approach

Optimization of the cultivation temperature

The solid phase fermentation of tomato cake has been performed in a wide range of temperature regimen – from 30°C till 50°C (with 5°C intervals) to establish the optimal temperature of cultivation.

Optimization of the composition of nutritional medium

The dtermination of the optimal amount of carbon source the cultivation of microscopic fungi was performed under the solid phase fermentation of different concentrations of the lignocellulosic substrate (2g; 4g; 6g; 8g)

The determination of optimal sourse of nitrogen performed under the solid phase fermentation of different sourse of the nitrogen. As the nirtogen mineral sources the following salts were used: NaNO3, and (NH4),SO4; HNO3 and $(NH4)_2HPO4$; various concentration of peptone, urease and yeast extract were used as the organic sources. Conditions under which the producer accumulated maximal amount of protein were regarded as optimal.

III. RESULTS

The purpose of the presented experiment was to receive the protein- and other physiologically active compounds-rich biomass by means of biotransformation of plant waste material with microscopic fungi. Nonpathogenic and nontoxic

strains of microscopic fungi: *Aspergillus terreus* A3; *Aspergillus fumigatus* G14; *Apsergillus versicolor* A4 *Apsergillus wentii* R12; *Sporotrichum pulverulentum* A32; *Trichoderma viride* R9; *Chaetomium cellulotycum* A43; *Chaetomium thermophile* A1, which were isolated from the ash-gray soils of the east Georgia forests and destructed wood of trees, were used as the bioconversion agents. As it known, a wide group of microorganisms, linked with the food chains, take part in wood destruction in the nature. These microorganisms step by step destruct the hard-digestible components of lignocellulose – cellulose and lignin. These polymers do represent the main components of the plant waste. By means of the waste biotransformation it is possible to receive the protein- and other physiologically active compounds-rich biomass. We supposed that among the microscopic fungi isolated from the destructed wood the lignocellulose-destructing strains would present [18]. On the initial step of experiments the cellulase and ligninase activities of the experimental microscopic fungi was performed to reveal the strains of above-mentioned activity (Table 1).

TABLE 1. Cellulase and ligninase Activities of microscopic fungi isolated from forest ecosystem

Microscopic fungi	Enzymatic activity,U/m	
	Cellulase	Ligninase
A.wentii R12	0.3	0
S. pulverulentum	0.55	12.8
T.a viride R9	0.35	0
C. cellulotycum	1.2	0
C. thermophile A1	0.05	0
A. terreus A3	0.2	0
As fumigatus G14	0.05	0
s versicolor A4	0.4	0
As fumigatus G14	0.05	0
s versicolor A4	0.4	0

Data of the Table 1 demonstrate that all experimental strains possess ability of cellulase synthesis at different levels. The best producer of the enzyme is *C. cellulotycum* A43.

As it was supposed, the ligninase activity was revealed only in *S. pulverulentum*A32, as the ability of delignification is characteristic only for this culture. It must be mentioned that *S. Pulverulentum* A32 reveals the high cellulase activity as well. Thus, two potential agents of lignocellulose waste bio-transformers were distinguished just at the initial step of investigation. Selection of a cheap and profitable raw material among the agro-industrial plant-waste, with high content of the main biopolymers (cellulose/hemicllulose), was the second task of the experiment. The chemical composition of some waste-products of the agricultural and food industry: apple- and tomato-cakes, citrus and orange peels, cereal's stubble (wheat and corn) and vine cuttings was studied for the evaluation of substrate's potential (Tabl 2)

TABLE 2. Chemical components of the waste-products of the agricultural and food industry (%, per dry weight)

waste-products	Water soluble extractive	Hemicellulose	Cellulose	Lignin
Apple cake	19.79	29.61	31.3	19.30
Tomato cake	17.60	29.70	33.7	19.00
Vine cuttings	18.50	29.50	24.0	28.34
wheat straw	15.20	29.6	35.5	19.71
Corn stubble	20.90	31.20	30.0	17.94
Orange peels	65.72	18.30	8.41	7.57
Citrus peels	65.43	18.74	8.76	7.07

From the table 2 is clear that except the peels of citruses, all tested substrates contained high amount of cellulose and hemicelluloses, which makes possible their application for biotransformation.

The screening of microscopic fungi under the solid phase fermentation of different plant-waste was performed on the

strains of microscopic fungi was the next step of our by microorganisms in the fermented substrat. (Table3)

TABLE 3. Screening of the active producer of protein under the solid phase fermentation of some waste materials of agricultural and food industry

Strain Substrate	Pure protein, %						
	Apple cake	Tomato cake	Vine cuttings	Wheat straw	Corn stubble	Orange peels	Citrus peels
Control	3.8	4.5	4.0	2.6	2.4	3.4	3.8
C.tcellulotycum A43	6.3	7.2	5.6	4.5	4.8	5.8	5.5
A.terreus A3	5.0	6.0	5.1	3.9	3.4	4.4	5.0
*S.pulverulentum*A32	5.2	6.4	5.4	4.0	4.2	4.9	5.5
A. fumigatus G14	4.5	5.2	4.7	3.7	3.2	4.7	5.1
*A .versicolor*A4	4.3	5.7	5.0	3.6	3.6	5.2	5.0
A.s wentii R12	4.6	5.0	4.9	4.2	4.0	5.3	5.2
T.viride R9	5.0	5.2	4.9	3.7	3.9	5.4	5.1
*C.thermophile*A1	4.9	5.7	5.0	3.8	4.0	5.5	5.0

According to the Table 3 it is clear that the tomato cake may be considered as one of the perspective substrates for fermentation; on the 8th day of its solid phase fermentation *C. Cellulotycum* A43 had

produced 7.2% proteins in the biomass. That is why the tomato cake was regarded as an appropriate substrate for producing a protein-rich biomass, and *C. Cellulotycum* A43 and *S. pulverulentum* A32 were selected as the active producers of protein-rich biomass. Though the last does not produce much protein in the biomass, but it is the only strain among the tested ones with lignin degrading ability. The tomato cake contains quite high amount of this hardly-degradable biopolymer.

Optimization of the cultivation conditions of the selected strains of microscopic fungi was the next step of our experiments. It is well known that duration of the cultivation process significantly influences the protein accumulation by microorganisms in the fermented substrat. From the fig.1 it is clear that both microscopic fungi were in lag phase during 2 initial days of cultivation; that's why there was no protein gain. Accumulation of the maximal amount of protein in the fermented substrate was mentioned on the 10-th day of solid phase fermentation; after both strains enter the stationary phase of growth. According to experimental data 10days of solid phase fermentation of the tomato cake was regarded as advisable for further experiments. Literary data demonstrate dependence of the metabolic activity of microorganisms on the cultivation temperature. The solid phase fermentation of tomato cake with the selected strains has been performed in a wide range of temperature regimen – from 30°C till 50°C (with 5°C intervals) to establish the optimal temperature of cultivation.

Experimentally was revealed that both microscopic fungi are thermophilic and prefer cultivation at 40°C (Fig.2).Thus, it was decided to perform the further bioconversion of the tomato cake at 40°C.

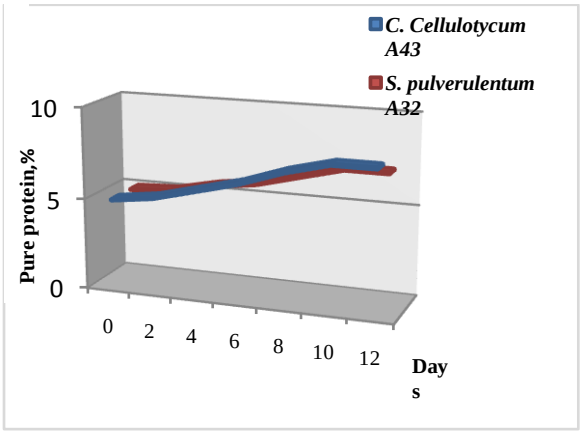

Fig. 1 The dynamics of protein accumulation by *C. Cellulotycum* A43 and *S. pulverulentum* A32 during the solid phase fermentation of tomato cake (experimental conditions: cultivation at 30°C; concentration of the substrate – 4g; pH of the nutritional medium – 5.5).

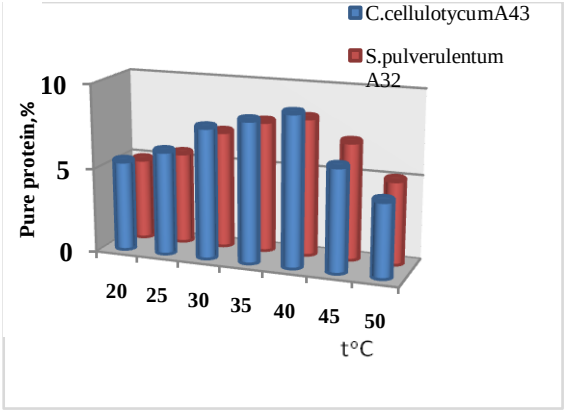

Fig. 2 The influence of cultivation temperature on the growth and development of *C. Cellulotycum* A43 and *S. pulverulentum* A32 (experimental conditions: concentration of the substrate – 4g; the duration of cultivation – 10 days; temperature range – 30°C – 50°C).

After the optimal conditions of cultivation (temperature and duration) were established for the selected strains, the optimization of the nutritional medium was aimed. Experiments to this direction were begun with the determination of the optimal amount of carbon source – the tomato cake. Accordingly, the cultivation of microscopic fungi was performed under the solid phase fermentation of different concentrations of the substrate(2g; 4g; 6g; 8g) (Fig. 3) Data of the Fig. 3 demonstrate that both strains of microscopic fungi accumulate the maximal amount of protein in the biomass when the nutritional medium contains 6g of the tomato cake. That's why the application of 6g of substrate is advisable in further experiments.

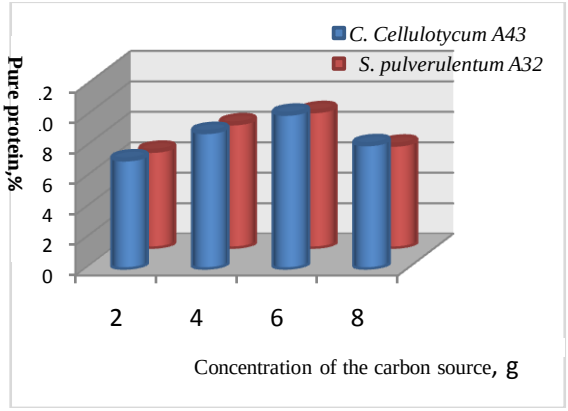

Fig.3. Dependence of the protein content in biomasses of *C. cellulotycum* A43 and *S.pulverulentum* A32 on different concentrations of the carbon source 2g; 4g; 6g;(experimental conditions: concentration of the substrate 8g; duration of the cultivation – 10days, at 40°C; pH of the nutritional medium – 5.5).

One of the significant components of the nutritional medium, which essentially affects microorganisms' growth and development and metabolic activity, is source of nitrogen. Selection of the optimal source of nitrogen for *C. Cellulotycum* A43 and *S. pulverulentum* A32 was the next step in our experimental work. Strains of experimental

microscopic fungi were cultivated on nutritional mediums containing different sources of nitrate - $NaNO_3$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, peptone, yeast extract and maltose extract. From Fig. 4 it is clear that *C. cellulotycum*A43 accumulated maximal amount of proteins (11.6%) in the biomass when the nutritional medium contained NH_4NO_3, while for *S. pulverulentum* A32 equally effective were mediums with NH_4NO_3, and $NaNO_3$. Accordingly, preparation of nutritional mediums with NH_4NO_3 was decided in further experiments. After the additional source of nitrogen was selected, the optimal concentration of the source (NH_4NO_3) for a particular strain has been established.

For these purpose strains of *C. Cellulotycum* A43 and *S. pulverulentum* A32 were cultivated on nutritional mediums with different concentrations of ammonium nitrate (15, 35, 75, 150, 200, 250, 300mg/6g substrate). According to experiments it was established that the strain of *C. Cellulotycum* A43 accumulated maximal amount of protein (13.5%) when the content of ammonium nitrate was 150 mg per 6g substrate, while under the same conditions *S. pulverulentum* A32 accumulated 10.0% of protein in the biomass.

Thus, protein-rich biomasses have been obtained on the base of optimization of the cultivation conditions and nutritional medium composition of *C cellulotycum* A43 and *S. pulverulentum* A32 applied in solid phase fermentation of tomato cake. Content of the protein in biomasses (13.5-10.0%) has been increased about 2.3-2.8 times, compared to initial substrate (4.9%).

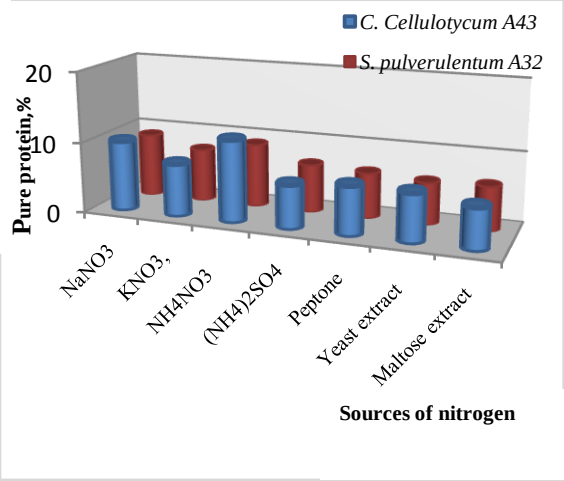

Fig.4 The content of pure protein in nutritional medium following the additional sources of nitrogen

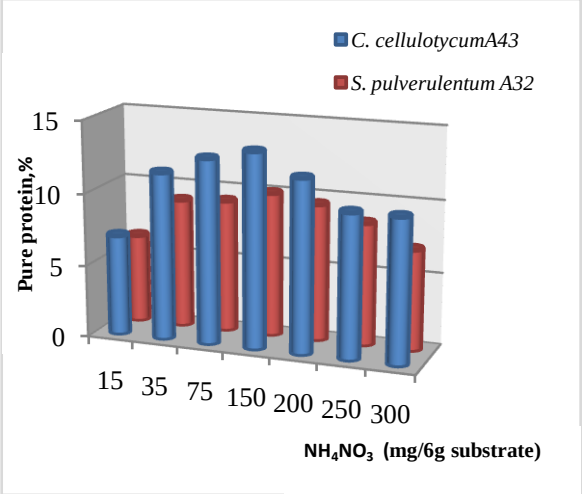

Fig. 5 Protein accumulation in the biomasses of *C. cellulotycum*A43 and *S. pulverulentum* A32 under different concentrations of the NH_4NO_3

Composition and extent of digestibility of the obtained biomasses was investigated fot the establishment of possibility of its application as food additive**.** As it was expedcted, thanks to lignolitic activity of *S.pulverulentum* A32 content of lignin in the fermented substrate was significantly decleaned; while *C.cellulotycum* A43 was not able to change the lignin component of the substrate et all. It is clear that the last strain of fungi is able to grow and accumulate protein on the expence of cellulose and hemicellulose degradation, which significantly decreases the extent of digestibility of the product **(**Tab**le4).**

TABLE 4. The solid phase fermentation of tomato cake with *C. cellulotycum* A43 and *S. pulverulentum* A32(experimental conditions: substrate – 6g; diration of the cultivation – 10days; temperature – 40°C; pH-5.5; ammonium nitrate – 150mg/6g substrate) (%, per dry weight

Microscopic fungi	Water soluble	Hemicelluloses	Cellulose	Lignin
Control	17.60	29.70	33.7	19.00
*C.cellulotycum*A43	19.5	20.0	22.5	38.0
S.pulverulentum A32	35.0	20.0	30.0	15.0

At the last step of experimental work the possibility of biotransformation of the tomato cake by the combined cultivation of *C. Cellulotycum* A43 and *S. pulverulentum* A32 has been studied, to increase the extent of the substrate bioconversion. (Table 5).

TABLE 5. The solid phase fermentation of tomato cake by the combined cultivation of *C. cellulotycum* A43 and *S. pulverulentum* A32 (%, per dry weight)

Microscopic fungi	Water soluble	Hemicellulos es	Cellulose	Lignin	Pure protein
*C.cellulotycum*A 43 + *S.pulverulentum* A32	47. 5	20. 0	20. 0	12. 5	16. 5

demonstrates that the content of lignin in the biomass, obtained by means of synergetic culture, was decreased till 12%; content of pure protein increased by 11.6%, compared to control; 42% of the soluble extractable substances are soluble sugars.

The obtained biomass is nonpathogenic, nontoxic and may be stored as dried powder, and used as the food additive

IV. CONCLUSIONS

1. The chemical composition of some agro-industrial waste – apple and tomato cake, vine cuttings, cereal and corn stubble, orange and citrus peels has been investigated. The possibility of their biotransformation by means of microscopic fungi has been established.
   Screening of microscopic fungi under the solid phase fermentation of different plant substrates has been conducted to reveal the active producer of protein. The profitable substrate for bioconversion – tomato cake, and two active producers of protein - *Chaetomium cellulotycum* A43 and *Sporotrichum pulverulentum* A32 were selected.
2. Protein-rich biomasses have been obtained on the base of optimization of the cultivation conditions and nutritional medium composition of the selected strains, where the content of protein was 2.3-2.8 times higher compared to initial substrate. The composition of the obtained biomasses has been determined.
3. The synergetic effect of the combined cultivation of *Chaetomium cellulotycum* A43 and *Sporotrichum pulverulentum* A32 on the biotransformation of tomato cake was demonstrated. Sugar (20%) and protein-rich (16.5%), easy-digestible, nontoxic and nonpathogenic biomass has been obtained on the base of combined cultivation of the selected strains under the optimal conditions, which may be used as a food additive.

REFERENCES

[1] Oliveira, M.D., Feddern, V., Kupski, L., Cipolatti, E.P., Bad iale-Furlong, E., & Souza-Soares, L.A. Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid- state fungal fermentation. Bioresource Technology, 2011; 102(17), 8335–8338

[2] Nigam P. S. nee' , Pandey A. Solid-State Fermentation Technology for Bioconversion of Biomass and Agricultural Residues, Biotechnology for Agro - Industrial Residues Utilisation 2009; 197-221

[3] Tesfaye A. Method of Standardization and Determination of Protein Extraction Using Orange Wastes by Aspergillus niger Isolate in Solid State Fermentation. The manuscript is submitted to Ethiopian Journal of Biological Sciences 2014; 13(2)

[4] Kupski, L., Cipolatti, E., Rocha, M., Oliveira, M.D., Souza-Soares, L.D.,& Badiale Furlong, E. Solid-state fermentation for the enrichment and extraction of proteins and antioxidant s, Int J Cur Res Rev; 2017; 9 (21) compounds in rice bran by Rhizopus oryzae. Brazilian Archives of Biology and Technology, 2012; 5(6), 937–942.;

[5] Schmidt, C.G., Goncalves, L.M., Prietto, L., Hackbart, H.S., & Furlong, E.B. Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus Rhizopus oryzae. Food Chemistry, 2014; 146, 371–377.

[6] Das, N., Mukherjee, M.,. Cultivation of Pleurotus ostreatus on weed plants. Biores. Technol., 2007; 98(14): 2723–2726

[7] Nasseri A.T. Rasoul-Amini S., Morowvat and Y.Ghasemi Single Cell Protein: Production and Process American Jurnal of Food Technology, 2011; 6(2):103-116

[8] Aggelopoulos, T.,. Katsieris K, Bekatorou A., Pandey A., Banat I.M and. Koutinas A.A. Solid state fermentation of food waste

[9] mixtures for single cell protein, aroma volatiles and fat production. Food chemistry. 2014; 145:710-716

[10] Jaganmohan, P., Purushottam B.and Prasad S.V.. Production of SCP with Aspergillus terrus using Solid State fermentation. Eur. J. Biol. Sci. 2013; 5(2):38-45

[11] Oshoma, Ge; Eguakun-Owe So, Conversion of Food waste to Single Cell Protein using Aspergillus Niger, J. Appl. Sci. Environ. Manage. 2018; 22 (3) 350 –355.

[12] FAO, Food and Agriculture Organization of the United Nations. Amino acid content of foods.Rome; 1970; 24.

[13] Ghose,T. K.; Measurement of cellulase activities. Pure andapplied chemistry 1987; 59(2); 257—268,

[14] Miller, G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 1959; 31, 426

[15] Tien M , Kirk T.K. Lignin-degrading enzyme from Phanerochaete chrysosporium: Purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proc Natl Acad Sci US A. 1984; 81(8): 2280–2284.

[16] Updegraff D.M. Semimicro determination of Cellulose in Biological materials. – Analit . Biochem., 1969, 32(2), 420-424

[17] Augustine O., Ayeni, Opeyemi, A., Adeeyo, Oyinlola M., Oresegun Temitayo, E. Oladimeji. Compositional analysis of lignocellulosic materials: Evaluation of an economically viable Appl method suitable for woody and non-woody biomass, American Journal of Engineering Research (AJER); 2015 4(4), 14-19.

[18] AOAC – Association of Official Analytical Chemists. Official methods of analysis (17th ed)

[19] Perinbam Kantharaj, Bharath Boobalan, Seeni Sooriamuthu, Ravikumar Mani, Lignocellulose Degrading Enzymes from Fungi and Their Industr