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Biotechnology

Bioconversion of Waste Products of French Fries and Chips into Protein-Rich Biomass

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For the purpose to obtain proteins and other bioactive compounds-rich biomass from the waste of Georgian production of french fries and chips the screening of *Sporotrichum pulverulentum* strains from the collection of microscopic fungi of the Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Geogia has been carried out. The strain *S. pulverulentum* G-11 has been selected. To increase the protein content in the obtained biomass, the optimization of cultivation conditions and nutrient medium composition of *S. pulverulentum* G-11under the solid state fermentation has been conducted. The optimal parameters for the cultivation of selected strain have been established. The possibility of bioconversion of the french fries and chips industry waste by means of *S. pulverulentum* G-11 has been demonstrated. Partly delignified, easily digestible protein-rich biomass is obtained. The content of lignin in the obtained biomass decreased by 38.76%, cellulose – by 41.1 % (which significantly raises the digestibility of biomass and its nutritional value), while the content of proteins increased by 13.5%, as compared to the control. © *2022 Bull. Georg. Natl. Acad. Sci.*

microscopic fungi, protein-rich biomass, solid-state fermentation, industry waste

The global level of hunger in the world is still dangerously high. According to the 2022 year' report of the world organization of food supply (SOFI) the number of hunger-disturbed people in 2021 reached 828 millions which approximately by 46 millions exceeds the pre-COVID-19 data.

Alarming is the FAO's forecast as well: since June of 2022 till September the lack of products will be worsened in 20 countries of the world. Food crisis was induced by the war in Ukraine, which significantly raised the world prices on food. Today about 45 million of the world population is on the edge of exhaustion and death because of starvation. Without special measures the life of millions of children will face the danger [1].

According to all above mentioned the scientific society of the world intensively searches for the alternative ways to recover the food deficiency. The massive industry of food or its particular components by means of non-traditional ways, on the base of microbial conversion of a cheap, row material (agricultural and food industry waste) is regarded as one of the convenient, safe and fast

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realizable technologies today for recovering the protein deficiency.

During the last period the Georgian market abundantly offers locally produced French fries and chips. The company "Freco" produces about 150 tones of these products annually. Approximately the same amount of the mentioned food is produced by another company – FRIXX. Both companies are supplied with the local, Georgian potatoes. Significant amount of the industrial remains is uselessly thrown and pollutes the environment. The microbial conversion of these residues has not conducted yet in Georgia. Accordingly the recycling of the waste will be profitable for fries and chips-making companies, which will produce bio-additives, rich in protein and biologically active compounds, together with the waste utilization.

Among the producers of biologically active compounds the microscopic fungi – one of the groups of mycelial fungi attract a special attention.

High permeability of the mycelium in the substrate, ability of synthesis of essential amino acids, semi-unsaturated linolic and linolenic acids, various vitamins and polysaccharides of pharmaceutical importance has turned this group of microorganisms into the effective producers of proteins and physiologically active substances. Their nutritional and taste properties are rated highly as well [2-5]. Accordingly the bioconversion of plant waste by means of microscopic fungi is considered as a real possibility to recover the food deficiency and protect the environment. The most significant role in this type of technologies belongs to the industrial strains. That is why selection of the appropriate strain, optimization of its cultivation conditions and elaboration of the principals for the conversion of industrial wastes into value added products is of great importance [6,7].

Materials and Methods

The nonpathogenic and nontoxic strains of *Sporotrichum pulverulentum* from the collection of microscopic fungi of Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia were applied in the study.

Screening of the fungi cultures for the conversion was performed under the solid-state fermentation (SSF) on the french fries and chips industry wastes during 8 days in the thermostat at 30°C. For this purpose 6 g of fully dried waste of plant origin, milled in 0.4-0.5 mm size particles was placed in conic 100 ml flasks and 12 ml of nutritional medium of the following composition was added (g/l): NH_4NO_3- 3.0; KH_2PO_4- 1.0; $MgSO_4.7H_2O$ – 0.5; $FeSO_4 . 7H_2O$-0.02. The pH of the nutrient medium was adjusted to 5.5-6.9 by means of 5% NaOH solution; the flasks were sterilized in an autoclave during 45min under one atmosphere. Two ml of fungal spores inoculum suspension was placed in cooled flasks.

After the cultivation, the content of flasks were transferred into previously weighted bottles, which were placed in thermostat at 105°C, to be dried till the permanent weight. The amount of biomass received after the bioconversion was calculated by the differences between the initial and final masses of bottles.

The main components of biomasses, received under the solid-state cultivation of microscopic fungi were determined gravimetrically: cellulose content in lignocellulosic substrates was determined according to Updegraff [8], hemicellulose was determined by weight method, treating sample with 0.1N H_2SO_4. The amount of lignin was determined by treating sample with 72% H_2SO_4 [9].

The percentage of raw protein was determined [10]. The optimization of nutrient medium composition and cultivation conditions of the selected strain was performed by the standard approach;

Table 1. Components of the wastes of Georgian production french fries and chips (% on dry weight)

Extracted	Hemicellulose	Cellulose	Lignin
39.1	26.9	15.5	18.5

Table 2. Screening of *S. pulverulentum* strains according to the accumulation of protein under the SSF of the waste of french fries and chips production (experimental conditions: cultivation at 30°C, substrate concentration – 6 g; pH of the nutrient medium – 5.5; the fermentation duration – 8 days)

	Culture	Raw protein, %
1	Control	12.5
2	*S. pulverulentum* SJ-35	14.8
3	*S. pulverulentum* N-2	14.2
4	*S.pulverulentum* G-13	15.0
5	*S.pulverulentum* G-11	16.0
6	*S.pulverulentum A-5*	14.4

conditions, under which as a result of SSF of the strain on the wastes the maximal amount of protein was produced was regarded as optimal.

Results and Discussion

The presented study aimed the biotransformation of waste from Georgian production of french fries and chips into protein- and other physiologically active substances-rich biomass. In order to achieve the goal, the strains of *Sporotrichum pulverulentum* isolated from the decomposed wood of different forest ecosystems of Georgia were selected as bioconversion agents. The selection of *S. pulverulentum* strains in our experiment was determined by the fact that among microscopic fungi, only this species is characterized by the ability to break down difficult-to-degrade biopolymer lignin, and that among them there would be lignocellulose decomposers.

The chemical composition of selected for the bioconversion substrate – waste of french fries and chips was investigated at the initial stage of experiment (Table 1).

As seen from Table 1, it is clear that the selected plant substrate contains enough amounts of cellulose and hemicellulose, and could be used for their application for bioconversion.

On the next step of investigations the screening of *S. pulverulentum* strains for their selection was

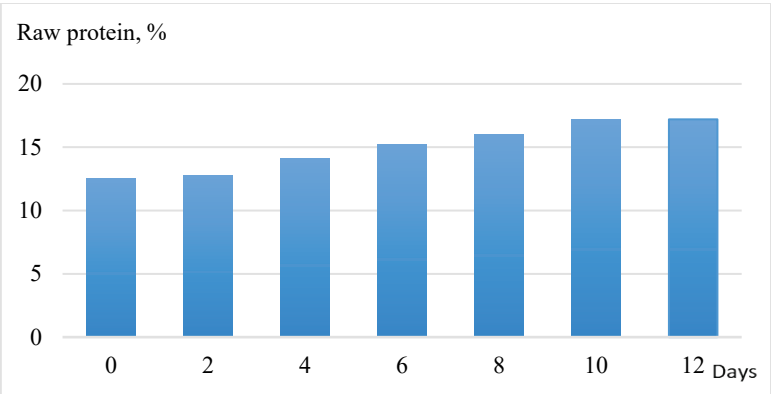

Fig. 1. Dynamics of protein accumulation under the SSF conditions of the waste of Georgian production of french fries and chips (experimental conditions: cultivation at 30°C, substrate concentration – 6 g, pH of the nutrient medium – 5.5).

performed under the solid-state fermentation on french fries and chips waste (Table 2).

Application of SSF in experiments was based on the speculation that by this method a lignocelluloses raw material is directly transformed, without any special type of bioreactors, aeration and additional energetic expenses, in contrary to submerged cultivation; correspondingly it is economically effective as well.

As seen from the Table 2, strain *S. pulverulentum* G-11 accumulated maximal amount of protein. To optimize the cultivation conditions of *S. pulverulentum* G-11 the dynamics of protein accumulation in relation with cultivation duration was investigated initially.

Fig. 1 shows that the maximal amount of proteins in the biomass produced by *S. pulverulentum* G-11 was accumulated on the 10th day of plant waste solid-state fermentation (17.2%). Accordingly it was reasonable to conduct the strain cultivation during 10 days.

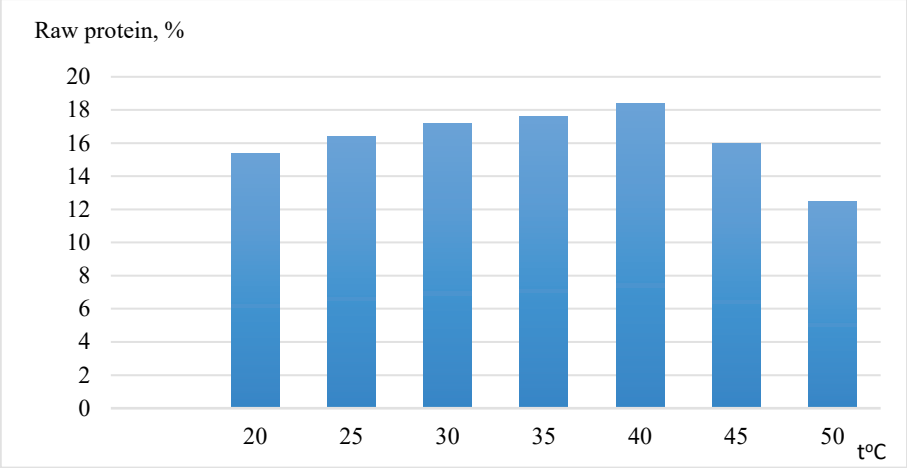

Fig. 2. Influence of cultivation temperature on growth and development of *S. pulverulentum* G-11 (experimental conditions**:** substrate concentration – 6 g, duration of cultivation – 10 days, temperature range – from 30°C to 50°C).

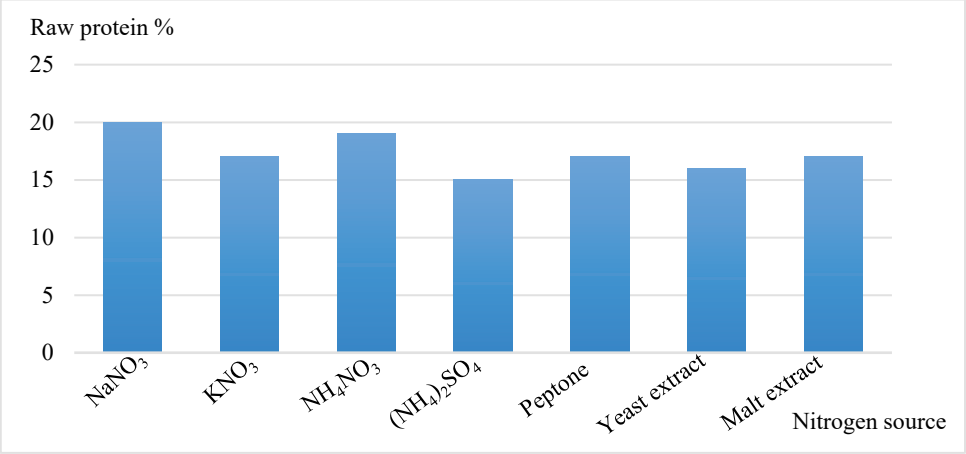

Fig. 3. Content of raw protein in biomass accumulated by *S. pulverulentum* G-11 at various additional sources of nitrogen (experimental conditions**:** substrate– 6 g, duration of cultivation – 10 days, temperature **–** 40°C; pH – 5.5; concentration of the nitrogen source – 35 mg per 6 g of substrate).

To establish the optimal temperature of *Sporotrichum pulverulentum* G-11 cultivation, SSF of french fries and chips waste was performed in the wide range of temperature – from 30°C to 50°C, with 5°C intervals**.**

As seen from the Fig. 2, *S. pulverulentum* G-11 is thermophile and prefers 40°C for cultivation.

As known, nitrogen souce is one of the important components of the nutrient medium, which essentially affects the growth and development of microorganisms and their metabolic activity. Therefore, on the next step of studies selection of the optimal source of nitrogen for *S. pulverulentum* G-11 cultivation was carries out. For this purpose the stain was cultivated at various sources of nitrogen – $NaNO_3$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, as well as peptone, yeast extract and malt extract containing nutrient mediums.

Fig. 3 demonstrates that during the SSF of french fries and chips waste *S pulverulentum* G-11 accumulated maximal amount of protein (24%) when sodium nitrate was added into the nutrient medium as an additional source of nitrogen. Accordingly it was reasonable to use $NaNO_3$ in further experiments.

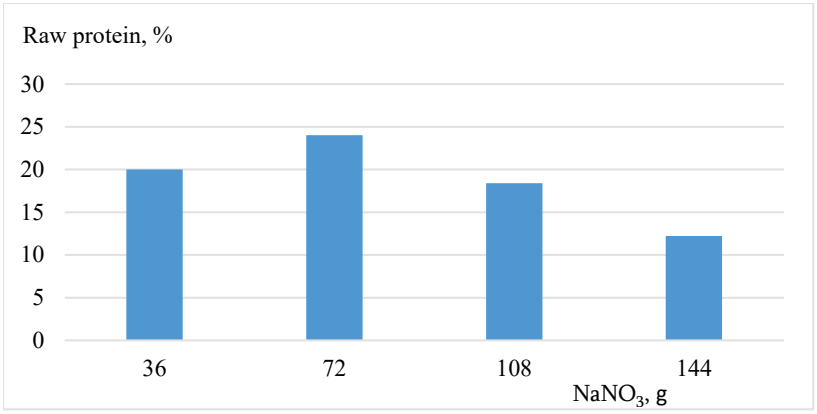

Fig. 4. Influence of different concentrations of additional nitrogen source on *S. pulverulentum* G-11 growth and development during the SSF of the waste of Georgian production of french fries and chips.

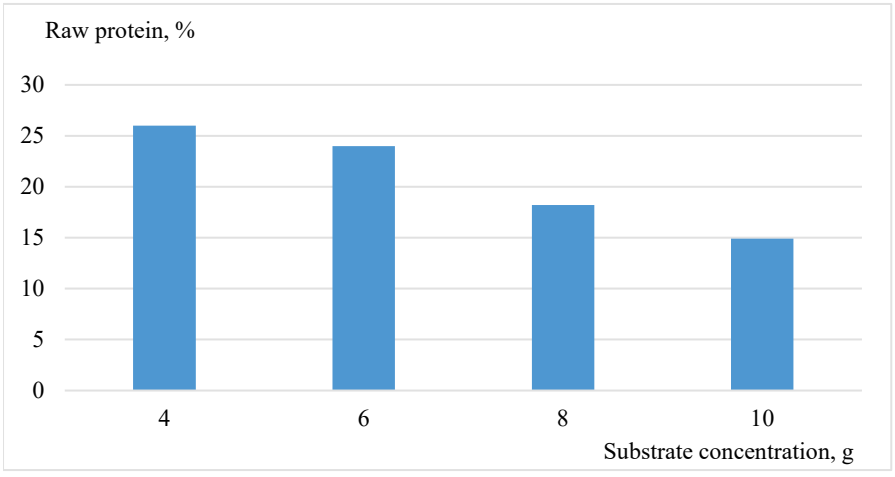

Fig. 5. Influence of different concentrations of carbon source on growth and development of *S. pulverulentum* G-11 during the SSF of the waste of Georgian production of french fries and chips.

On the next step of experiments the influence of different concentrations of the selected nitrogen source on growth and development of *S. pulverulentum* G-11 has been studied (Fig. 4). The results clearly demonstrate that when the concentration of $NaNO_3$ was 72 mg per 6 g of substrate the experimental culture accumulated maximal amount of protein. Further increase of the nitrogen in the medium caused evident inhibition of strain's growth (Fig. 4).

After the optimal concentration of additional source of nitrogen was established the influence of different concentrations of fermented substrate – french fries and chips waste on growth and development of *S. pulverulentum* G-11 has been investigated. The highest amount of protein was received when the amount of substrate was 4 g (Fig. 5).

Thus, on the base of consequent experiments which comprised 10 days SSF of french fries and chips waste and aimed the optimization of cultivating conditions and nutrient medium composition of *S pulverulentum* G-11, was obtained the biomass where the content of protein was by 13.5% higher, as compared to control.

To establish the possibility of application of the obtained biomass as additive for animal feeding, the extent of its digestibility and composition has been studied. As seen from the Fig. 6, content of hardly degradable bio-polymers in the accumulated biomass decreased: lignin – by 38.76%, and cellulose – by 41.1%.

This indicates that the coefficient of digestibility of the product has significantly improved.

The obtained biomass is nonpathogenic, nontoxic; it may be stored dried, as powder and may be recommended as an additive in animal feeding.

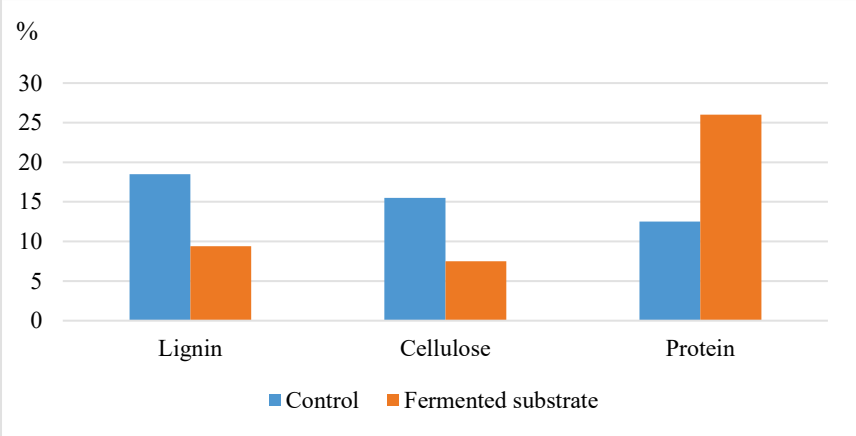

Fig. 6. Content of hardly degradable biopolymers (cellulose and lignin) in biomass obtained during the SSF of the waste of Georgian production of french fries and chips.

Conclusions

1. The chemical composition of the waste from Georgian production of french fries and chips has been studied. The possibility of bioconversion of the waste into protein-rich biomass by means of microscopic fungus *S. pulverulentum* – G-11 has been established.

2. On the base of optimization of cultivation conditions and nutrient medium composition for *S. pulverulentum* - G-11 easily digestible and partly delignified, protein-rich biomass has been obtained; in particular, 10 days SSF of fries and chips waste resulted in accumulation of biomass by *S. pulverulentum* – G-11, in which the content of hardly degradable bio-polymers was decreased:

lignin - by 38.76%, and cellulose – by 41.1%, while the content of proteins increased by 13.5%, compared to the control.

3. The obtained biomass is nonpathogenic, nontoxic, and may be stored dried, as powder; it is recommended as additive in animal feeding.

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ბიოტექნოლოგია

კარტოფილი ფრისა და ჩიფსების წარმოების ნარჩენების ბიოკონვერსია ცილებით მდიდარ ბიომასად

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კარტოფილი ფრისა და ჩიფსის წარმოების ნარჩენებიდან ცილითა და სხვა ბიოაქტიური ნაერთებით მდიდარი ბიომასის მიღების მიზნით,საქართველოს აგრარული უნივერსიტეტის დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტის მიკროსკოპული სოკოების კოლექციაში არსებულ *Sporotrichum pulverulentum-ის* შტამებს შორის ჩატარებულია სკრინინგი. შერჩეულია აქტიური შტამი *S. pulverulentum* G-11. ბიომასაში ცილის შემცველობის გაზრდის მიზნით, განხორციელებულია *S. pulverulentum* G-11-ის კულტივირების პირობებისა და საკვები არის შემადგენლობის ოპტიმიზაცია მყარფაზოვანი კულტივირების პირობებში. დადგენილია შერჩეული შტამის კულტივირების ოპტიმალური პარამეტრები. ნაჩვენებია *S. pulverulentum* G-11-ით კარტოფილი ფრისა და ჩიფსის წარმოების ნარჩენების ცილით მდიდარ ბიომასად კონვერსიის შესაძლებლობა. მიღებულია ადვილად მოსაწელებადი, ნაწილობრივ დელიგნიფიცირებული, ცილით მდიდარი ბიომასა, რომელშიც ლიგნინის შემცველობა 38,76%-ითაა შემცირებული, ცელულოზის 41,1 %-ით, ხოლო ცილის შემცველობა კონტროლთან შედარებით 13,5%-ით არის გაზრდილი, რაც მნიშვნელოვნად ზრდის ბიომასის მონელებადობის ხარისხსა და კვებით ღირებულებას.

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