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Bioconversion of Grape Juice Production Residues into Protein- and Functional Ingredient-rich Biomass - Waste-free Technology Development Perspective

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***Abstract*— *A protein- and bioactive compound-rich biomass was obtained through the deep fermentation of grape juice production residues by* Aspergillus terreus *IK40. The digestibility and nutritional value of the product obtained through bioconversion was studied. The crude protein content in the biomass is 19.0%*, *which fully corresponds to the recommended level for pig feeding during the active growth phase. The crude fat content (7.6%) also satisfies pig feeding standards. The biomass is rich in easily metabolizable sugars (141 mg/g), which plays a significant role in energy balance formation and promotes microbial fermentation in the pig digestive tract. The digestibility level of the biomass is improved due to the reduction of hard-to-degrade biopolymer content. The product is rich in micro and macro elements, contains biologically active metabolites (ascorbic acid, tocopherol, carotenoids, and proline) and possesses high total antioxidant activity. Thus, the obtained biomass represents not only a nutrient-rich but also a functionally valuable product, which application in pig feed will contribute to improving growth parameters and strengthening the organism's natural immune barriers.*

***Keywords*— bioconversion, mycoprotein, digestibility of the biomass, antioxidant activity, functional food supplement**

I. INTRODUCTION

Georgia is a country with unique climate and agrobiodiversity, which has centuries-old traditions of grapes and viticulture cultivation (Ekhvaia and Akhalkatsi, 2006; Imazio et al., 2013). This creates a solid foundation for the development of grape-derived products industry, including juices. Currently, several enterprises operate throughout the country, producing high-quality natural grape juice, which ensures both the satisfaction of domestic demand and the growth of export potential. The amount of bio-waste generated during juice production increases year by year. These residues (grape skins, stems, pomace, and other granular ballast material) are often discarded into the environment without utilization, which causes soil biochemical imbalance and microbiological contamination. At the same time, according to the global practice, grape juice and, in general, wine production residues have high potential for use in the food industry, pharmaceutical, cosmetic, and biotechnological sectors, due to their content of polyphenols, flavonoids, dietary fibers, fatty acids, and other bioactive compounds (Maicas, 2020; Hoxha, Lennartsson & Taherzadeh, 2025).

Obviously, interest is growing in biotechnologies that ensure the removal of potential pollutants from the environment and the bioconversion of grape juice production residues into food additives of high nutritional value. Intensive research is being conducted using microscopic fungi *Aspergillus spp.*, *Trichoderma spp.*, *Rhizopus spp.* and other strains, which transform food industry waste into protein-rich biomass or extracts enriched with bioactive components (Sadh et al., 2018; Gmoser et al., 2020; Umashankar & Nygård, 2024)**.**

The integration of these processes into waste-free, ecologically clean, and economically efficient technologies is particularly significant. Against the backdrop of these global trends, the development and implementation of innovative technologies for biotransformation of agro-industrial waste (including grape juice production residues) becomes relevant in Georgia.

Based on series of experiments conducted at the Biotechnology Laboratory of the Georgian Technical University, mycoprotein-enriched biomass was obtained under conditions of deep cultivation of the microscopic fungus *Aspergillus terreus* IK40 on grape juice production residues. Determination of the product's non-toxicity and its nutritional value evaluation was the aim of the presented study.

II. RESEARCH OBJECT AND METHODS

The strain *Aspergillus terreus* IK40 from the collection of mycelial fungi at the Georgian Technical University served as the research object. Residues of grape juice production from the Saperavi variety **(GJPR)** were used as a biotransformation substrate. The substrate was dried in a thermostat at 80°C, after which it was ground in a laboratory mill into small-sized (0.5-1.0 mm) particles. The obtained flour was stored in hermetic containers at room temperature till analysis.

Deep fermentation of GJPR proceeded under previously established, optimal for the strain conditions: at 37°C, on a shaker at 180 rpm for 8 days. For this purpose, 4 g of GJPR absolute dry weight (ADW) was weighed into 250 ml Erlenmeyer conical flasks, to which 100 ml of nutrient medium of optimal composition, previously selected for the strain, was added (g/L): $NaNO_3$ - 9.0; KH_2PO_4 - 2.0; $MgSO_4 \times 7H_2O$ - 0.5; $FeSO_4 \times 7H_2O$ - 0.02. The initial pH of the nutrient medium was 5.5.

The flasks were sterilized by autoclaving at 1.2 atm, 120°C for 45 minutes. After cooling, the flasks were inoculated with 2.5 ml of fungal spore suspension.

After completion of cultivation, the flask contents were centrifuged at 4000 rpm for 5 minutes, and the precipitate was dried in a thermostat at 105°C. The main biopolymers - lignin and cellulose - were determined in the biomass obtained by deep cultivation of mycelial fungi (Van Soest et al., 1991). The biomass was also analyzed for: easily metabolizable sugars (Miller, G.L. (1959), using dinitrosalicylic acid reagent; crude protein (after Kjeldahl) (AOAC, 2005), crude fat (Soxhlet method) (AOAC, 2005), moisture (oven drying method at 105°C) (AOAC, 2005), magnesium (Atomic Absorption Spectrophotometry) (AOAC, 2005), zinc (Atomic Absorption Spectrophotometry) (AOAC, 2005), phosphorus (colorimetric method using vanadate-molybdate) (AOAC, 2005), ash (muffle furnace at 550°C) (AOAC, 2005), potassium (flame photometry) (AOAC, 2005); *as* well as for Ascorbic acid (Ermakov et al., 1987), tocopherol (Fillipovich et al., 1982), carotenoids (De Carvalho et al., 2012) and proline (Bates et al., 1973) content and total antioxidant activity (Koleva et al., 2002). The toxicity of the strain was tested using the Brine shrimp lethality assay (BSLA) (Mosmann, 1983).

Each experimental series were repeated three times. The tables and figures present the average values of the results and their standard deviations.

III. RESULTS AND DISCUSSION

The present study represents a logical continuation of experiments conducted at the **Biotechnology** Laboratory of the Georgian Technical University. Its purpose was the biotransformation of grape juice production residues into functional feed additives for agricultural animals using *Aspergillus terreus* IK40. Such an integrated approach to fruit juice production waste management today is considered as an essential condition for forming low-waste production and sustainable agrosystems (Aguilar et al., 2002; Pandey et al., 2000).

According to literary data some strains of *A. terreus* are characterized by the ability to produce mycotoxins (Frisvad et al., 2004). Since the biomass obtained through GJPR biotransformation is intended for use as an additive in animal feed rations, ensuring safety was essential. This was accomplished using the Brine shrimp lethality assay, which is considered a rapid and informative test for biotoxicity assessment (Meyer et al., 1982; Solis et al., 1993). The express test revealed the non-toxicity **of *A. terreus* IK40** and created the basis for further research. After excluding toxicity, an assessment of the product's digestibility level was carried out.

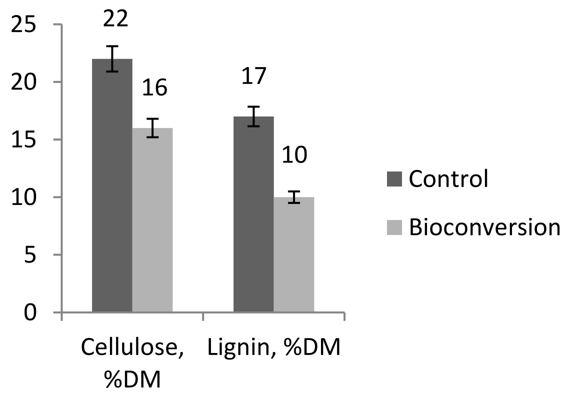

Fig. 1 Content of hard-degradable biopolymers in products obtained by conversion of grape juice production residues

From the obtained results is clear that in the biomass obtained through 8-day fermentation of GJPR, cellulose content decreased by 6%, while of lignin - by 7% (on dry matter basis) (Fig. 1). Cellulose is considered as moderately digestible component for monogastric animals, especially pigs, whose digestive tract allows partial microbial degradation of fiber with the help of intestinal microflora (Jurgens et al., 2002; Bach Knudsen, 2001). The reduction of lignin content to 10% gives the biomass additional advantages, since this hardly degradable biopolymer of phenolic origin significantly reduces the degree of digestibility (Van Soest, 1994).

At the next stage of the research, the content of main macro- and microelements, some vitamins, fats, and easily metabolizable sugars was determined in the target product, and its antioxidant potential was also assessed (Tables 1 and 2).

The obtained data demonstrate that the crude protein content in the product obtained by bioconversion fully corresponds to the recommended level for pig feeding during the active growth phase (NRC, 2012); especially when the protein component is accompanied by easily metabolizable carbohydrates. The high content of easily metabolizable sugars (141 mg/g) plays a significant role in forming the energy balance and promotes microbial fermentation in the digestive tract of pigs (Fuller & Chamberlain, 1990; Montagne et al., 2003); as well the obtained amount of fat is considered optimal for pig feed from the perspective of improving energy value (Whittemore, 1993) (Table 1).

Table 1 Content of macro- and micro elements, easily metabolizable sugars, raw fat and proteins in products obtained by conversion of grape juice production residues

Component	Content, %
Moisture	6,1
Sodium	0,95
Zinc	0,002
Phosphorus	0,43
Magnesium	0,20
Potassium	0,92
Easily metabolizable sugars	14,1
Raw fat	7,6
Ash	4,8
Raw protein	19, 0

The antioxidant profile of the product prepared by biotransformation deserves particular attention (Table 2): significant levels of ascorbic acid, tocopherol, carotenoids, and proline were established in the biomass. These biomolecules act as stabilizers of cellular protective systems, especially during periods of stress, vaccination, or other negative impacts (Surai, 2002). For example, tocopherol (vitamin E) is known as a supporter of normal reproductive function and the immune system (López-Bote et al., 2001), while proline stabilizes cellular structure under osmotic stress conditions (Szabados & Savouré, 2010).

The 70.4% of total antioxidant activity points to strong bioactivity (Table 2). Similar indicators have been recorded only in certain plant extracts that are used as natural enzyme additives in animal feed (Koleva et al., 2002).

Thus, the obtained biomass represents not only a nutrient-rich but also functionally valuable product, which application in pig feed will promote improvement of growth parameters and strengthening of the organism's natural immune barriers, which is crucial for modern approaches to sustainable and antibiotic-free livestock farming.

Table 2 Content of antioxidants and total antioxidant activity of products obtained by conversion of grape juice production residues

Component	Content
Ascorbic acid	71.4 mg/%
Tocopherol	8.2 mg/g
Carotenoids	60.9 mg/%
Proline	388 µg/g
Total antioxidant activity	70.4%

Based on the conducted research, we can conclude:

1. The biomass obtained through bioconversion of grape juice residues by *Aspergillus terreus* IK40 represents a functional feed additive enriched with proteins and energy components for agricultural animals, which not only ensures nutritional balance but also contains natural antioxidants, giving it strategic importance in ecologically safe animal feeding.
2. Microbial conversion of grape juice production residues with *Aspergillus terreus* IK40 is not only an ecologically justified process but also provides a promising source of nutritional additive. The technology fully corresponds to sustainable development principles and promotes the formation of low-waste production in the agricultural sector.

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