A New Functional Food Additive - Biomass Obtained by Bioconversion of Apple Juice Production Waste

Zakariashvili Nino[1]; Khokhashvili Izolda[2*]; Kutateladze Lali[3]; Urushadze Tamar[4]; Jobava Maya[5]; Tsiklauri Nino[6]; Burduli Tamar[7]; Khvedelidze Rusudan[8]; Aleksidze Tinatin[9]

Sergi Durmishidze institute of Biochemistry and Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia
*Corresponding Author: Izolda Khokhashvili

Received:- 27 April 2025/ Revised:- 05 May 2025/ Accepted:- 12 May 2025/ Published: 31-05-2025
Copyright @ 2025 International Journal of Environmental and Agriculture Research
This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted Non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract**—** *Functional food products rich in bioactive compounds (protein, lipids, vitamins, etc.) are especially demanded and popular in the modern food market. Due to its easy availability and low cost, apple juice production waste, which is produced in thousands of tons in Georgia, can be considered as a promising raw material for the production of functional food. Mycoprotein-enriched, easily digestible, non-toxic biomass was obtained as a result of successive experiments carried out in the biotechnology laboratory of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia. The product obtained by microbial conversion contains Na, P, Ca and Mg, from as macro elements, and also is rich in Zn. The content of raw protein in the biomass is 18%, which fully corresponds to the standards of protein content in chicken feed for s. The raw fat content in a biomass is 7%. The amount of cellulose in the product corresponds to the standards as well. The product was found to have moderate antioxidant activity (60-70%). Based on all above mentioned, the biomass made on the basis of submerged fermentation of apple juice production waste can be recommended as a feed additive in poultry diet.*

Keywords**— Apple juice industry waste, Mycelial fungi, Protein-rich biomass, Submerged fermentation, Functional food.**

I. INTRODUCTION

Global climate change and related environmental problems require the development of new approaches and concepts for sustainable development of the environment. One of the possible ways to solve the mentioned problem is a new economic model - circular bioeconomy, which allows the bioconversion of a colossal amount of waste generated in agro-industry.

Unpredictable population growth, massive urbanization and industrialization, as well as a high-protein diet, have all caused protein consumption to an exponential increase in recent years. Experts anticipate that pressure from demographic, societal, and economic reasons will lead to protein consumption to decline even further in the near future. Conflicts around the world, climate change, increased food prices, poor nutrition, a lack of protein, and micronutrients, among other factors, will make the problem of food shortage worse [1],[2],[3]. Based on this, the global scientific community is working intensively to find alternate solutions to the food scarcity. Nowadays, one of the most convenient, safe, and rapidly implemented technologies for replenishing food protein deficiency is the biotransformation of cellulose-containing raw materials (agricultural and food industry wastes) into protein-rich biomass and various valuable secondary metabolites of microbial origin [4].

Filamentous fungi attract special attention among the mass producers of valuable biologically active compounds of plant origin [5]. This group of microorganisms is regarded as an efficient producer of protein and physiologically active compounds due to their high ability to penetrate the substrate via their mycelium, the ability to synthesize some essential amino acids, semi-unsaturated linoleic and linolenic acids, various vitamins, and polysaccharides with pharmaceutical properties. In addition, they are highly valued in terms of nutrition and palatability [6],[7],[8].

Functional food products, rich in bioactive substances (proteins, lipids, vitamins, etc.) are especially demandable and popular at the market. Because of its easy reach and low cost the waste of apple juice industry, produced in thousand tons in Georgia, may be regarded as a perspective raw material for functional food industry. Together with the utilization of the waste, food supplements rich in protein and biologically active compounds may be prepared by the microbial conversion of the mentioned waste, which would make the industry even more profitable [9],[10].[11].

The microscopic fungus *Fusarium venenatum* is regarded as the most effective producer of commercial mycoprotein today. Due to its fast growth, filamentous structure, high protein content in biomass, absence of pigments, odors and toxins the fungus is recognized as the best producer of alternative protein.[12],[13][14]

The bioconversion of plant-origin waste with fungi is viewed as a real chance to alleviate the food scarcity and preserve the environment in accordance with all of the aforementioned points. Certainly, in this kind of technology, the industrial strain is crucial [15],[16]. Therefore, it is especially critical to find a potential perfect producer, optimize its cultivation conditions and develop the technological bases for the production feed additive [17],[18].

To reveal an active protein producer among the fungi of the genus *Fusarium* in the collection of microscopic fungi of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia and bioconversion of apple juice industry waste into mycoprotein-rich biomass and other bioactive compounds with the selected strain, was the aim of the presented research.

II. RESEARCH OBJECT AND METHODS

2.1 Object of the study:

The non-pathogenic and non-toxic strains of the genus ***Fusarium*** from the mycelial fungi collection of the Durmishidze Institute of Biochemistry and Biotechnology of the Agrarian University of Georgia was the object of the study.

The residue of Georgian apple juice production (AJPW) was used as a biotransformation substrate.

2.2 fermentation of the AJPW:

The submerged fermentation of the AJPW (before the optimization of the composition of the nutrient medium) was carried out on a shaker, 180/rpm, at 30ºC, for 10 days. For this purpose 6 g of the absolute dry (ABW) waste(*Cole-Parmer® LB-200 Analytical Balances)*, ground in a laboratory mill (*RRH-A1000,High-speed Multi-function Comminutor*) into small particles (0.4-0.5 mm) was weighed and placed in 250 ml Erlenmeyer conical flasks. It was added 50 ml. of the following composition nutrient medium (g/l): $NaNO_3$- 3.0; KH_2PO_4 - 1.0; $MgSO_4x7H_2O$ - 0.5; $FeSO_4x7H_2O$-0.02; the pH of the medium was adjusted to 6.5 with 0.5% solution of NAOH (*pH-meter MT30266628, FP20*. Flasks were sterilized by autoclaving at 1 atm. for 45 minutes. Cooled flasks were inoculated with 2 ml of fungal spore suspension. After the cultivation was completed, the content of the flasks was centrifuged for 5 min, 4000 rpm; the sediment was placed in a beaker brought to constant weight, and dried in a thermostat at 105ºC to a constant weight.

2.3 Determination of biomass composition:

The nutritional value of biomass was determined by modern standard methods: crude protein content according to ISO-5983-2: 2009/2016; crude fat - GOST 5668-68; humidity -GOST 13496 3-92; sodium - EN1551102017; magnesium - EN1551102017; zinc - EN 1551102017; phosphorus - EN 1551102017; ash by GOST 13979 6-69; potassium - EN 1551102017. The content of soluble sugars was determined by spectrophotometric method, using 3,5-*dinitrosalicylic acid* (Spectrophotometer SPECOL 11, Carl Zeiss, Germany. Total antioxidant activity was determined using diphenylpicrylhydrazyl (DPPH) [19].

2.4 Optimization of growing conditions and the composition of a nutrient medium:

Optimization of the nutrient medium composition and cultivation conditions of the selected strain was carried out according to the standard approach [20]. The cultivation conditions in which the producer accumulated the maximum amount of protein were considered optimal.

Reagents used. In the experiment, reagents from Sigma-Aldrich were used.

III. RESULTS AND DISCUSSION

High protein content in the biomass and fast growth of the strain are considered the main parameters in the selection of the industrial producer of mycoprotein [21]. Screening among the fungi of the genus Fusarium from the collection of microscopic fungi of Durmishidze Institute of Biochemistry and Biotechnology of the Georgian Agrarian University under the conditions of AJPW submerged fermentation was conducted, in order to select an effective agent for the bioconversion of AJPW. The strain Fusarium spp. S2 was found to accumulate the maximum amount of protein in the biomass on the eighth day of cultivation (Table 1). That is why it was considered appropriate to use this strain for further research.

TABLE 1

Screening of the protein active producers among the microscopic fungi of the genus Fusarium under the submerged fermentation conditions of AJPW (experimental conditions: temperature of cultivation 30ºC, concentration of the substrate – 6g; pH of the nutrient medium – 6,5; duration of the fermentation – eight days)

Strain of the microscopic fungus	Content of a true protein, %
Fusarium spp. NP-7	4.7±0.8
Fusarium spp. A6	5.6±0.8
Fusarium spp. S2	6.4±1.0
Fusarium spp. S12	4,2±0.7
Fusarium spp. M11	3,8±0.4
Fusarium spp. M28	3,9±0.4
Fusarium spp. D18	3.8±0. 4

It is known that the growth and development of microorganisms significantly depends on the strain cultivation conditions; in particular, on pH, duration of cultivation and temperature. The optimization of cultivation conditions of *Fusarium* spp. S2 was started by determining the optimal duration of cultivation. For this purpose, the dynamics of protein accumulation by the selected strain was studied, depending on the duration of cultivation.

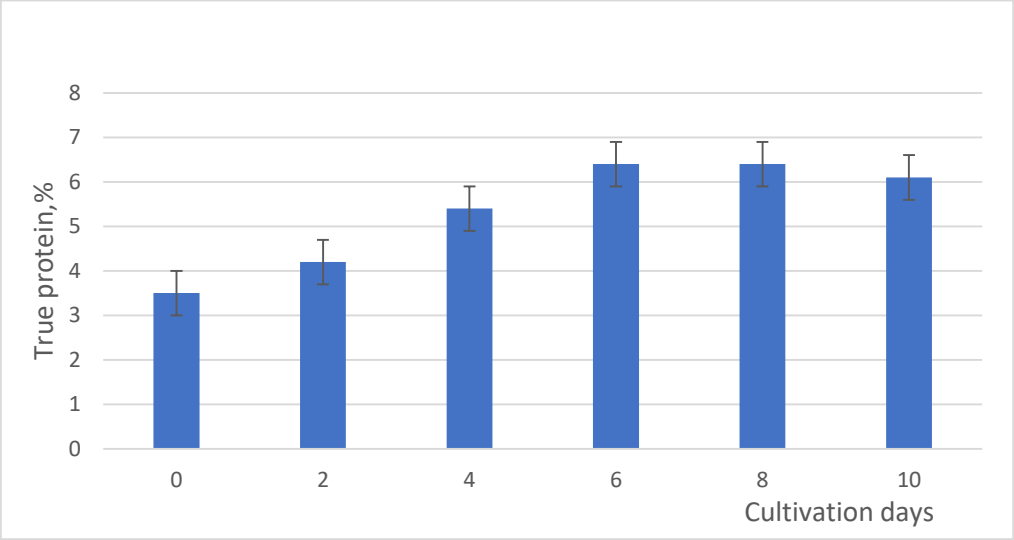

FIGURE 1: The dynamics of protein accumulation under the submerged cultivation conditions of *Fusarium* spp. S2 on AJPW (experimental conditions: cultivation T - 30ºC; substrate concentration - 6g; nutrient medium pH - 6.5).

From the Fig.1 is clear that under the submerged fermentation of AJPW *Fusarium* spp. S2 accumulates maximum amount of protein on the sixth day; that is why it was considered appropriate to conduct cultivation of the strain during six days in further experiments.

To determine the optimal temperature of cultivation of *Fusarium* spp. S2 the submerged fermentation of the AJPW was conducted in wide range of temperature – 25ºC – 50ºC (with intervals of 5ºC).

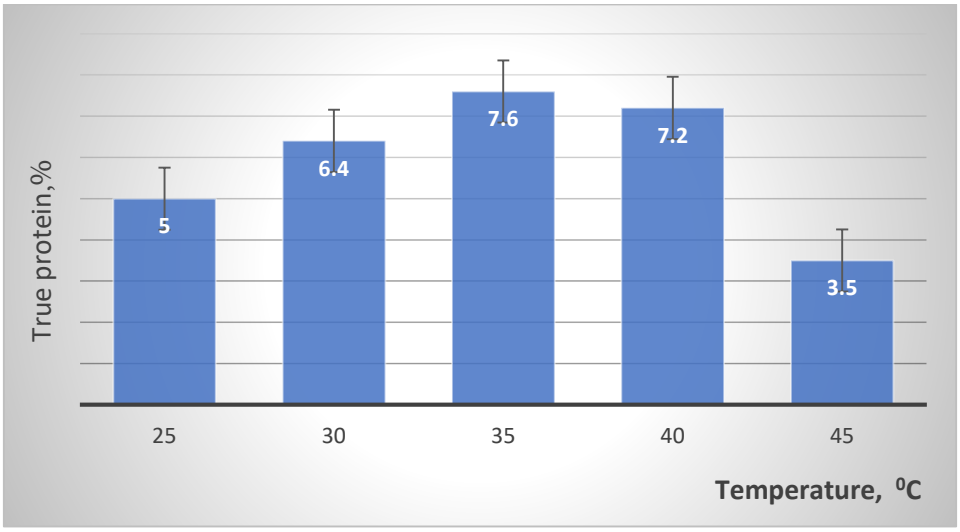

FIGURE 2: Influence of the cultivating temperature on the growth and development of *Fusarium* spp. S2 (experimental conditions: concentration of the substrate – 6g; pH of the nutrient medium – 6,5; duration of the fermentation – six days, temperature range 30ºC – 50ºC)

Fig. 2 demonstrates that *Fusarium* spp. S2 is a mesophylle and prefers cultivation at 35**ºC** that is why for further investigations this temperature was regarded as the optimal for substrate bioconvertion.

One of the significant parameters, which essentially affect the metabolic activity of microorganisms, is pH of the nutrient medium [18]. Accordingly at the next stage of the experiment the influence of pH of the nutrient medium on growth and development of *Fusarium* spp. S2, as well as on protein accumulation in the biomass was studied.

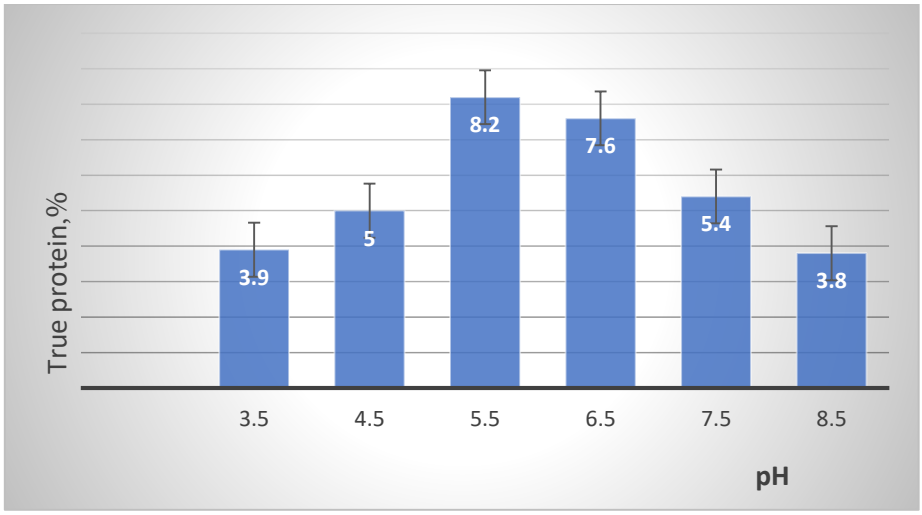

FIGURE 3: Influence of the initial pH of the nutrient medium on protein accumulation in the biomass of *Fusarium* spp. S2 (experimental conditions: concentration of the substrate – 6g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – from 3,5 till 8,5 (with 1,0 intervals))

From the Fig. 3 is evident that during the submerged fermentation on AJPW *Fusarium* spp. S2 accumulated maximum amount of protein (8,2%) when the initial pH of the fermentation area was 5,5. Accordingly it was decided to proceed submerged fermentation of AJPW with *Fusarium* spp. S2 under pH 5,5 conditions.

It is well known that the efficiency of the bioconversion of plant origin substrate significantly depends on the nature and concentration of carbon source. That is why on the next stage of experiment the optimal concentration of AJPW – the bioconversion substrate - was determined. For this purpose different concentrations of the substrate (2, 4, 6, and 8grams) were taken for the submerged fermentation.

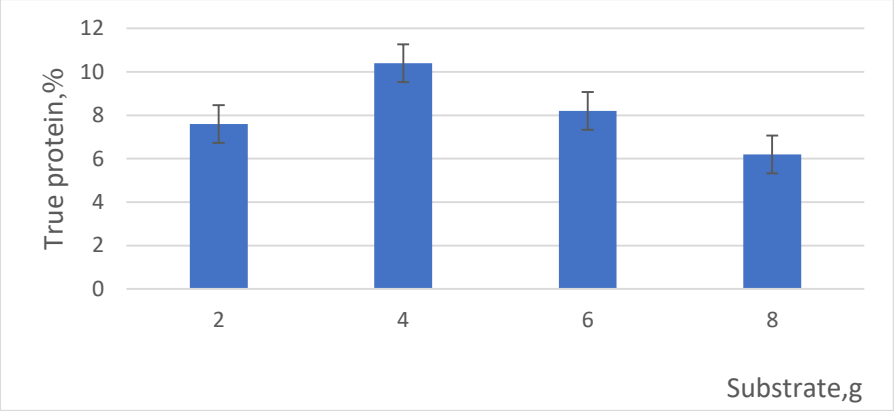

FIGURE 4: Influence of the different concentrations of carbon source on the protein accumulation in *Fusarium* spp. S2 biomass (experimental conditions: concentration of the substrate – 2, 4, 6 and 8g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5)

From the Fig. 4 is clear that *Fusarium* spp. S2 accumulates maximum amount of protein while cultivated on 4g of the substrate. That is why in further experiments the submerged cultivation of *Fusarium* spp. S2 was conducted on 4g of the substrate.

For the purpose to increase the content of protein in the biomass selection of the optimal source of nitrogen for *Fusarium* spp. S2 was aimed on the next step of study.The selected strain was cultivated on peptone, yeast and malt extract containing nutrient mediums with different sources of nitrogen – $NaNO_3$, KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$.

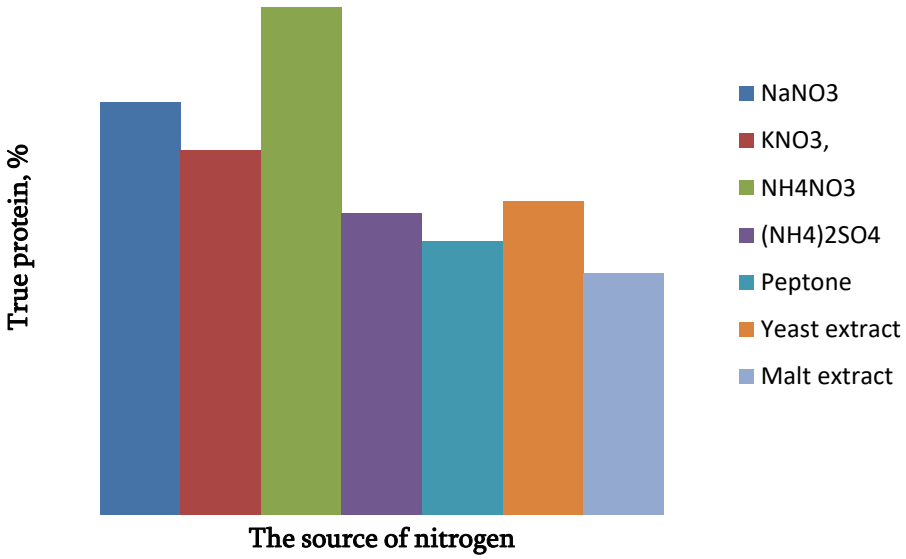

FIGURE 5: Content of true protein in *Fusarium* spp. S2 biomass following additional sources of nitrogen (experimental conditions: concentration of the substrate 4g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5; organic and inorganic sources of nitrogen with concentration 108g/4g of substrate)

As it is clear from the Fig. 5 *Fusarium* spp. S2 accumulates maximum amount of protein when NH_4NO_3 is used as an additional source of nitrogen in the nutrient medium. That is why this salt was used in further experiments.

Determining the optimal concentration of NH_4NO_3 was the last step in the optimization of *Fusarium* spp. S2 cultivating conditions.

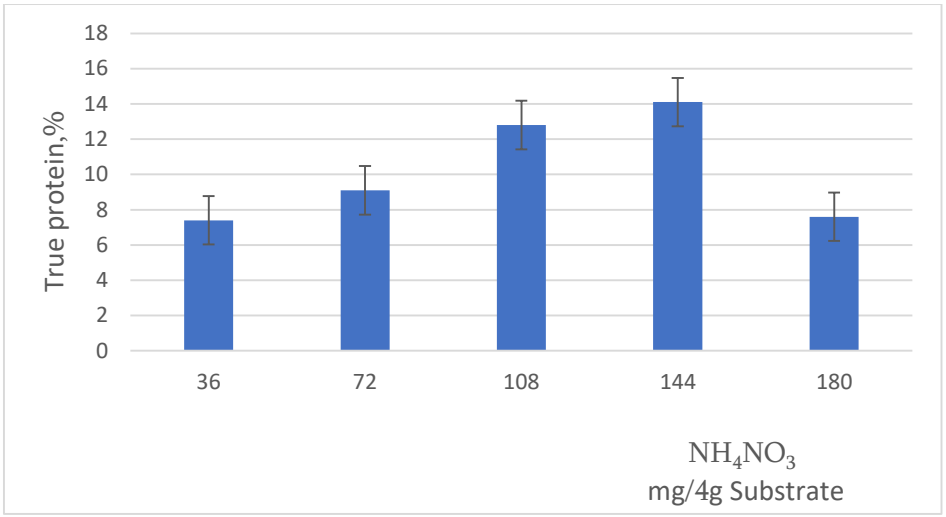

FIGURE 6: Influence of different concentrations of nitrogen source of protein content in *Fusarium* spp. S2 biomass (experimental conditions: concentration of the substrate 4g; duration of the fermentation – six days, temperature 35ºC pH of the nutrient medium – 5,5; NH_4NO_3 concentrations: 36mg, 72mg, 108mg, 144mg and 180mg per 4g of the substrate)

Fig. 6 clearly demonstrates that the fungal strain accumulated maximum amount of protein when concentration of NH_4NO_3 in the nutrient medium was 144mg per 4g of substrate.

Thus, according to series of subsequent experiments on the base of bioconversion of AJPW by means of *Fusarium* spp. S2 was obtained enriched with mycoproteins, easily digestible biomass, where the content of protein was 4times higher, compared to the initial substrate. The nutrient value of the prepared potential food additive was determined at the last step of experiment.

TABLE 2
Content of micro and macro elements, true protein and crude fat in AJPW bioconversion (with *Fusarium* spp. S2) products (calculated on absolutely dry weight (ADW)

	Components	Content in biomass, ADW
1	Humidity	4,2±0.8
2	Sodium	1,0%±0.2
3	Zinc	11 mg /kg±1.2
4	Phosphorus	0,29%±0.2
5	Ash	3,3%±0.4
6	Magnesium	0,28%±0.02
7	Potassium	0,70%±0.03
8	True protein	14,1%±1.2
9	Crude fat	6,5 %±1.0
10	Total antioxidant activity	65%±1.5

From the Table 2 is clear that the product of microbial conversion contains Na, P, Ca and Mg, as microelements and is rich in Zn as well. The crude fat in the biomass is 6,5%, which is higher than the norm determined for combined feed of egg laying hens. The product has moderate antioxidant activity (60-65%).

The fact that FAO annually implements a number of political activities to improve the market access and investment for these products to enhance market access and investment for these products as well as support the competitiveness of small and medium-sized businesses illustrates the urgency of the production of such alternative sources of protein[22].

According to all above mentioned the biomass obtained as a result of bioconversion of AJPW by means of *Fusarium* spp. S2, may be recommended as a food additive in poultry feed.

IV. CONCLUSIONS

1) Possibility of biotransformation of AJPW into protein- and other biotic compounds rich biomass by means of microscopic fungus *Fusarium spp.* S2 has been established.
2) As a result of the optimization of submerged cultivation conditions and nutrient medium composition of *Fusarium* spp. S2 the true protein content of the biomass obtained from the biotransformation of AJPW increased from 3.5% to 12.8%.
3) Biomass is rich in crude fat, micro- and macro elements, and has antioxidant properties; accordingly it can be recommended for use as a feed additive in poultry diet.

REFERENCES

[1] Henchion M., Hayes M., Mullen A.M.,Fenelon M. and Tiwari B.- Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. Foods. 20; 6(7):53. 2017 doi: 10.3390/foods6070053
[2] FAO, The Global Network Against Food Crises: 5- 30, 2022,
[3] Rachel A. Bahn, Sibelle El. Labban. Food Security and Nutrition Leveraging nutrition for food security: the integration of nutrition in the four pillars of food security.; In book: Food Security and Nutrition; Publisher: Elsevier. 2021.
[4] Tim J. Finnigan A. Benjamin TWall, Peter J Wilde, Francis B Stephens, Steve L Taylor, and Marjorie R. Freedman. Mycoprotein: The Future of Nutritious Nonmeat Protein; Symposium Review; 3(6): nzz021, 2019
[5] Pedro F. Souza Filho. Advances in Food and Nutrition Research, Vol.101, P. 153-179 Chapter Five - Fungal protein, 2022. https://doi.org/10.1016/bs.afnr.2022.04.003
[6] Hashempour Baltork F., Khosravi-Darani K., Hosseini H., et al. Mycoproteins as safemeat substitutes. Journal of Cleaner Production, v.253, 2020.https://doi.org/10.1016/j.jclepro.2020.119958
[7] Yabaya A., Saleh A. Ado.. Mycelial protein production by *Aspergillus niger* using banana peels. Science World Journal, 3(4):9-12, 2010. DOI:10.4314/ swj. v. 3i4.51819
[8] 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.
[9] Baiano, A. Recovery of biomolecules from food wastes—A review. Molecules 19, 14821–14842, 2014.
[10] Tsegaye, B.; Jaiswal, S.; Jaiswal, A.K. Food Waste Biorefinery: Pathway towards Circular Bioeconomy. Foods 10, 1174, 2021. https://doi.org/10.3390/foods10061174
[11] Reshab Majumder,a Saptadip Miatur,b Akash Sahac and Shamim Hossain, Mycoprotein: production and nutritional aspects: Sustainable Food Technology,2,81, 2024, DOI: 10.1039/d3fb00169e
[12] Changtai Zhang, Xin Guan, Shiqin Yu, Jingwen Zhou, Jian Chen,Production of meat alternatives using live cells, cultures and plant protein Current Opinionin Food Science, 43: 43-52.2022, https://doi.org/10.1016/j.cofs.2021.11.002
[13] Shiqin Yu, Weizhu Zeng, Sha Xu, Jingwen Zhou, Expediting the growth of plant-based meat alternatives by microfluidic technology: identification of the opportunities and challenges Current Opinion in BiotechnologyV. 75, 2022. 102720 https://doi.org/10.1016/j.copbio.2022.102720
[14] Colosimo R., Mulet-Cabero A.I., Warren F.J., Mycoprotein ingredient structure reduces lipolysis and binds bile salts during simulated gastrointestinal digestion. Food Funct, 2020; 11:10896-10906
[15] Aggelopoulos T., Katsieris K., Bekatorou A., Pandey A., Banat I.M and Koutinas A.A. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. Food chemistry, 145:710-716, 2014.
[16] Tyler J. Barzeea, Lin Caob, Zhongli Panb, Ruihong Zhangb, Fungi for future foods, Journal of Future Foods;; 1-1: 25-37, 2021.
[17] Nasseri, A.T., Rasoul-Amini, S., Moromvat, M.H., Ghasemi, Y. Production of single cell protein from fruits waste. Am. J. Food Technol., 2011; 6(2):103-116
[18] Kahlon S.S, Dass S.K. Biological convertion of paddiy straw into feed. Biol.Wastes. 1987; 22:11-21
[19] Irina I. Koleva, Teris A. van Beek, Jozef P. H. Linssen, Aede de Groot, Lyuba N. Evstatieva, Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. Phytochemical Analysis.V 13(1) :. 8-17, 2002. https://doi.org/10.1002/pca.611
[20] Oshoma, Ge; Eguakun-Owe So. Conversion of Food waste to Single Cell Protein using *Aspergillus Niger*, J. Appl. Sci. Environ. Manage.;22 (3): 350 –355, 2018.
[21] Maria Papagianni. Advances in citric acid fermentation by *Aspergillus niger*: biochemical aspects, membrane transport and modeling Biotechnol. 25(3):pp. 244-263, 2007. 10.1016/j.biotechadv.2007.01.002).
[22] Research EU, FOOD 2030: Innovative EU research ensures food system is future-ready. Results Pack on Food Systems Transformation, Luxembourg. Publications Office of the European Unio. 2018.