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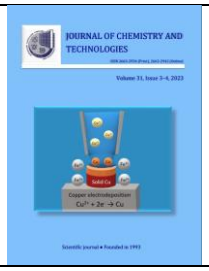

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STUDY OF THE PRODUCTIVITY OF PLEUROTUS OSTREATUS MUSHROOM CULTIVATION ON WASTE COFFEE GROUNDS TREATED WITH ALFALFA EXTRACT

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Abstract

The aim of the study was to evaluate the possibility of using coffee grounds (CG) for the production of several target products - nutritious mushrooms of the genus *Pleurotus ostreatus* and a protein supplement to the daily diet of farm animals, as well as to assess the effect of alfalfa extract on the main indicators of mushroom cultivation. Six different substrate formulas of coffee sludge (CS) / wheat straw with CS content of 100 %, 80 %, 70 %, 60 %, 50 % and 0 % were investigated using weight, chemical and physicochemical methods. On substrates with higher CS content, the mycelial growth rate and total colonisation time were longer and increased with increasing CS content. The addition of CS did not have a significant effect on the composition of *Pleurotus ostreatus* mushrooms. The treatment of substrates with alfalfa extract accelerated the colonisation period by 2–3.1 days, and the activation of the stages of complete colonisation of the substrate by mycelium after treatment with alfalfa (*Medicago sativa)* extract was 14.96–26.56 %. The high proportion of CS in the substrate leads to a decrease in biological efficiency and yield, while the treatment of substrates with alfalfa extract increases biological yield, economic yield and biological efficiency. The nutritional value of mushrooms was improved mainly on substrates with 50 % CS content compared to fruits cultivated on substrates without coffee sludge.

Keywords: recycling; coffee waste; ecobiotechnology; composting; cultivation; Pleurotus ostreatus mushrooms; waste substrate; feed additive, coffee sludge

ВИВЧЕННЯ ПРОДУКТИВНОСТІ ВИРОЩУВАННЯ ГРИБІВ *Pleurotus ostreatus* НА ВІДПРАЦЬОВАНІЙ КАВОВІЙ ГУЩІ, ОБРОБЛЕНІЙ ЕКСТРАКТОМ ЛЮЦЕРНИ

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Анотація

Метою дослідження була оцінка можливостей використання кавової гущі (ГК) для виробництва поживних грибів роду *Pleurotus ostreatus* та білкової добавки до щоденного раціону сільськогосподарських тварин, а також впливу екстракту люцерни на основні показники вирощування грибів. Було досліджено шість різних формул субстрату кавовий шлам (КШ)/пшенична солома з вмістом КШ: 100 %, 80 %, 70 %, 60 %, 50 % і 0 % хімічними та фізіко-хімічними методами. На субстратах з вищим вмістом КГ швидкість росту міцелію та загальний час колонізації були довшими і зростали зі збільшенням вмісту КГ. Додавання КГ не мало значного впливу на склад грибів *Pleurotus ostreatus*. Обробка субстратів екстрактом люцерни прискорювала період колонізації на 2–3.1 доби, а активація стадій повної колонізації субстрату міцелієм після обробки екстрактом люцерни становила 14.96–26.56 %. Високий вміст КГ у субстраті призводить до зниження біологічної ефективності та врожайності, а обробка субстратів екстрактом люцерни підвищує біологічну врожайність, економічну врожайність та біологічну ефективність. Поживна цінність грибів була покращена переважно на субстратах з 50 % вмістом КГ порівняно з плодами, вирощеними на субстратах без кавового шламу.

Ключові слова: переробка; кавові відходи; екобіотехнологія; компостування; культивування; гриби *Pleurotus ostreatus*; відпрацьований субстрат; кормова добавка, кавовий шлам.

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Introduction

Coffee is a beverage made from the roasted seeds of the coffee tree. Today, the coffee business industry has become a global industry. According to the International Coffee Organisation, more than 9.98 million tonnes of coffee were consumed in 2020, and the coffee trade around the world continues to develop and grow steadily. According to statistics, the annual consumption of coffee by the global population is more than 500 billion servings of coffee drinks per year [1]. Numerous studies have been conducted to confirm its significant benefits for the adult human body in moderate consumption [2; 3].

As the popularisation of various coffee drinks spreads around the world, the coffee industry is actively developing, with its core business being technological processes, the final stage of which is coffee drinks in one form or another [4; 5]. As of today, the world has rightly recognised the value of secondary raw materials generated as a result of these processes. After all, only about 20 % of the coffee fruit is used in the production of coffee drinks, and the remaining 80 % is made up of secondary raw materials [6; 7]. the accumulation of which leads to a negative impact on the environment. More than 28,000 tonnes of coffee industry waste are generated annually [8]. This includes various coffee industry wastes: cascara, patchmantle, silver cobwebs, and coffee sludge [9; 10]. The largest share of them is coffee sludge, which is generated at enterprises producing instant coffee or other coffee drinks, as well as at restaurant businesses in the preparation of various coffee drinks [11]. As a rule, small and medium-sized businesses do not use such waste, but simply dispose of it. The main, most popular methods of coffee sludge disposal are landfilling or incineration. However, these methods of disposal are quite inefficient and unpromising, and they also cause extremely high environmental damage. When coffee grounds end up in a landfill, they begin to decompose, producing methane, a greenhouse gas that is more than 20 times more potent than carbon dioxide in contributing to global warming. They are also potential sources of dangerous pathogens that can contaminate groundwater. Combustion produces particulate matter that adversely affects the air quality in the vicinity [10; 12].

Today, the global scientific community is actively studying possible methods of coffee waste disposal. Many new technologies have been created that have a number of advantages and disadvantages. It has been proven that due to its composition, coffee sludge can be used in many industries.

One of the most promising methods of coffee sludge utilisation is biotechnology through the cultivation of oyster mushrooms (*Pleurotus ostreatus*) on a coffee sludge substrate [13]. A variety of mushrooms can be grown when utilising plant waste through mushroom cultivation. However, due to a number of its advantages, it is the culture of oyster mushroom (Pleurotus ostreatus) that is the most successful choice for cultivation. It is one of the most productive and technologically advanced cultivated edible mushrooms. Oyster mushrooms (*Pleurotus ostreatus)* are the second largest producer of mushrooms in the world [14]. They are important in commercial production because of their organoleptic properties and nutritional value. In addition to being well balanced in proteins and vitamins and being a high-calorie product [15] oyster mushrooms contain a set of biologically active substances that are of great pharmacological interest due to their antibiotic, immunomodulatory, and anticancer effects [16; 17].

As a saprophyte, the fungus grows on dead wood and other lignocellulosic substrates [18; 19]. Since coffee grounds consist of a high percentage of cellulose, hemicelluloses and lignin [20], they can potentially be used as an additional or alternative substrate for the cultivation of Pleurotus species. In addition, it is known that Pleurotus fungi can detoxify and remove phenolic compounds from industrial and domestic wastewater by producing extracellular laccase and peroxidase secretions [21]. Hypothetically, the cultivation of these fungi on substrates containing coffee grounds could lead to a decrease in the phenol and caffeine content of the waste substrates. In other words, the cultivation of Pleurotus fungi can produce protein-rich food and act as a mycorrhizal process to remove phytotoxins in substrates containing coffee grounds. This will allow the spent substrate to be used as an additive to cattle feed or as a fertiliser for plants. There are studies on stimulating the productivity of Pleurotus ostreatus mushrooms with bioflavonoids of plant origin, as well as increasing the C/N ratio, which affects the growth rate of the mycelium [22; 23].

The aim of the study was to evaluate the possibility of using coffee grounds (CG) for the production of several target products – nutritious mushrooms of the genus *Pleurotus ostreatus* and

a protein supplement to the daily diet of farm animals, as well as to assess the effect of alfalfa extract on the main indicators of mushroom cultivation.

Material and Methods

Fresh wheat straw and seed alfalfa were obtained from a local private farm, Shabo (Ukraine, Odesa region). The CS consisted of waste generated during the brewing of coffee at the last stages of preparation in a local restaurant Zucchini (Ukraine, Odesa). It was collected with the help of employees who placed the CS in special collection containers. Mushroom caviar Pleurotus Ostreatus was purchased in an amount of 1 kg from TM "Seeds of the Country" at the Greenwood Trading House (Ukraine, Kyiv).

Wheat straw was chopped to a particle size of 4 cm using a small-sized universal feed chopper MUIK-10 and soaked for 3 hours in containers with water at a straw: water ratio of 1 : 20. Excess water was squeezed out manually. In order to prevent the development of fungi, competitors were subjected to heat treatment with boiling water at a weight ratio of substrate: water of 1 : 20. Cooling took place for 5 hours, after which the water was separated by decantation.

The coffee grounds were pasteurised in the coffee brewing process and used within 24 hours of collection. Therefore, no further heat treatment of the coffee grounds was carried out.

The alfalfa was ground to a particle size of 3 cm. Next, the extractant (5% NaCl solution) in distilled water was added and subjected to extraction by heating in a water bath at a weight ratio of 1 : 6 in a refluxing refrigerator for 3 hours to a bioflavonoid content of 67.54 mg/g of extract in terms of luteolin-7-glycoside.

Next, the substrates were prepared. The prepared straw was added to the coffee sludge and mixed. The substrate was activated by adding an aqueous extract of sowing alfalfa in a weight ratio of 1 : 0.5.

The studied substrate formulas are shown in Table 1. A mixture of substrates consisting of 100 % straw (C6) was used for comparison. Treatment C1 was also tested, completely replacing straw with CS, to assess whether such a replacement was possible. The moisture content of the substrate mixture was maintained at about 70–75 % at pH= 7.5...9.0, and the substrate temperature was room temperature.

Table 1

Substrate formulas based on dry weight

Substrate name	Coffee sludge (*% w/w*)	Wheat straw (*% w/w*)
C1	100.0	–
C2	80.0	20.0
C3	70.0	30.0
C4	60.0	40.0
C5	50.0	50.0
C6	–	100.0

The treatment of substrates with alfalfa extract was also investigated (Table 2).

Table 2

Substrate formulas based on dry weight

Substrate name	Coffee sludge (*% w/w*)	Wheat straw (*% w/w*)	Alfalfa water extract in relation to the substrate *(% w/w*)
C7	100.0	–	50
C8	80.0	20.0	50
C9	70.0	30.0	50
C10	60.0	40.0	50
C11	50.0	50.0	50
C12	–	100.0	50

Five hundred grams of homogenised substrates were packed in transparent plastic bags and 25 g of mycelium was added (at the rate of 5 % by weight of the substrate). The inoculated mycelium was mixed evenly with the substrate, the ends of the film were tied, and holes were made on the sides to ensure gas exchange. Incubation was performed in complete darkness at a temperature of 23–25 °C and high relative humidity of 80–90 %. No ventilation was performed, as excess carbon dioxide promotes mycelial growth.

After spawning, the mushroom blocks were transferred to a room with a temperature of 8–10 °C and natural light. Relative humidity of up to 95 % was maintained manually by periodic spraying using a sprinkler system to promote fruiting body development. Relative humidity and temperature were monitored using a thermo-hygrometer.

Mycelial growth was measured every two days. The time to pin head initiation, time to the first and second harvest waves were also noted. It was expressed in the number of days after sowing with mycelium. The production was evaluated at the level of each treatment by determining the number and weight of bunches (g/bag-1), number and weight of mushrooms (g/bag-1), biological yield (BY) (g/bag-1), economic yield (EY) (g/bag-1), biological efficiency (BE) (%). EY is the weight of weighed fruiting bodies of Pleurotus ostreatus mushrooms after removal of the stem bases [24]. BE – the ratio of fresh weight of mushrooms to the initial dry weight of the substrate. All treatments were carried out in triplicate.

Crude protein was calculated by recalculating the amount of total Nitrogen using a coefficient of 4.38 for mushroom biomass and 6.25 for substrates [25]. The content of total Nitrogen was determined by the Kjeldahl method. The following catalysts were used for the ashing of 0.5 g of crushed samples: 0.5 g of K_2SO_4 and 0.125 g of CuO were used for ozonation by heating in a mineraliser. The Nitrogen content (%) was determined by titration with a 0.1 n NaOH solution neutralised with 0.1 n HCl after ammonia distillation [26].

For substrates, crude fibre content was determined using the enzymatic gravimetric method of analysis, and for fungi, based on standard methods of approximate analysis [27].

The Antron method was used to determine the total carbohydrate content of substrates and mushrooms [26].

The crude fat content was studied by extraction with petroleum ether (for mushroom biomass) and hexane (for substrates) in a Soxhlet apparatus [28].

The total amount of soluble sugars for mushrooms and substrates was determined by high-performance liquid chromatography.

The mineral composition of the substrates was analysed using atomic absorption spectrophotometry [29]. In the dried samples of the respective mushrooms, the same minerals were measured using inductively coupled plasma atomic emission spectrophotometry after extraction of the element in a 0.1N acidic HCl solution.

Determination of fatty acid profile in fungal and substrate samples. The preparation was placed in a glass ampoule and 1.0 ml of 2N acetyl chloride solution in pre-cooled methanol was added. The mixture was kept at 80 °C for 4 hours. The fatty acid methyl esters were extracted with hexane. The n-hexane fraction was injected into a chromatograph. The analysis of fatty acid methyl esters was performed on an Agilent 6890N/5973 inert chromatography-mass spectrometry system (Agilent technologies, USA). HP-5MS capillary column, length 30 m, inner diameter 0.25 mm, phase thickness 0.25 μm. Temperature range 150–250 °C, temperature gradient 4 °C/min, carrier gas helium, flow rate through the column 1.0 mL/min. Evaporator temperature 250 °C, with a flow split of 1 : 100. The ionisation mode was electron impact with an energy of 70 eV, and the chromatogram was obtained in SCAN mode in the range of 40–700 m/z. The identification of the components of the studied samples was carried out using the NIST02 mass spectrum library and a standard mixture of fatty acid methyl esters.

The total phenolic content was determined in the substrates before and after the cultivation process. The analysis was performed using the methods described in [30]. Substrate extracts of 0.2 ml were mixed with 1.5 ml of 10 % Folin-Ciocalteu reagent. Next, 1.2 ml of 7.5 % (w/v) Na2CO3 solution was added and incubated at room temperature in the dark for 30 minutes. The absorbance at 765 nm was measured using a microplate reader (Tecan Infinite® 200 Pro, Switzerland). The total phenolic content was expressed as mg of gallic acid equivalents per 100g of dried sample. The caffeine content in the substrates was monitored using high-performance liquid chromatography (HPLC) [31].

The quantitative results obtained in the experiments were processed by statistical methods of analysis and the arithmetic mean and confidence intervals were calculated using the computer program Microsoft Excel 2010 (USA). The reliability of the research results was assessed according to the Student's t-test at the 5 % significance level in the OriginPro 8.5.1 software (USA). The data were presented as mean ± standard deviation.

The research scheme is shown in Fig. 1.

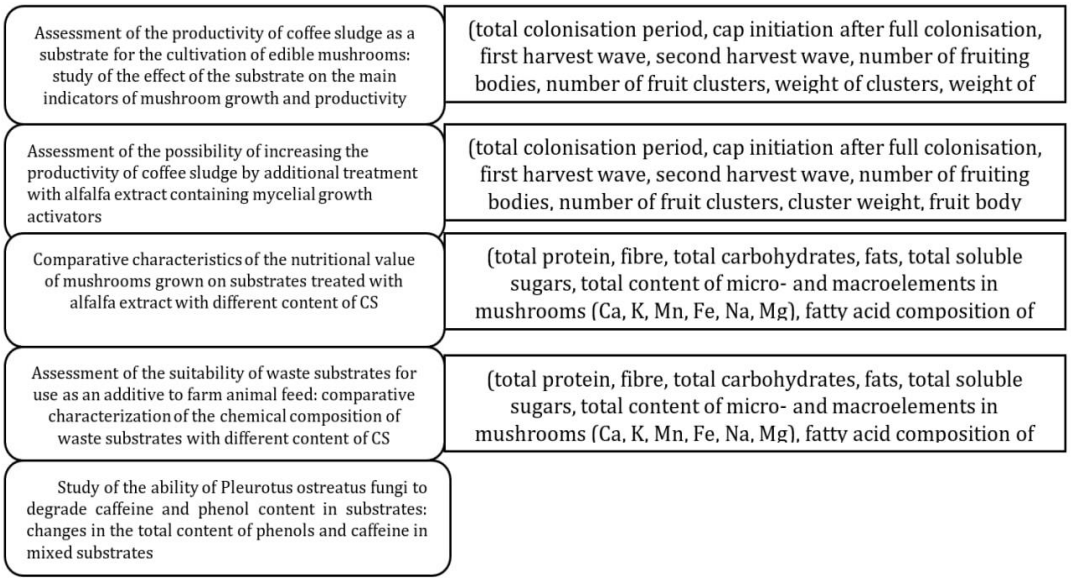

Fig. 1. Scheme of the research

Results and Discussion

Effect of substrate on mushroom production and composition.

Table 3 shows the effect of substrate formulations on the production properties of mushrooms cultivated on these substrates.

Table 3

Effect of substrate on the main indicators of mushroom growth and production

Main characteristics	C2	C3	C4	C5	C6
Total colonization periods, *DAMS**	20.3±0.19	17.7±0.2	15.4±0.34	14.5±0.33	14.2±0.19
Pin head initiation after full colonization, *DAMS**	27.4±0.34	24.2±0.3	20.1±0.4	19.3±0.33	19.2±0.2
First wave of harvest, *DAMS**	32.4±0.14	28.5±0.1	24.2±0.2	23.2±0.14	23.8±0.25
Second wave of harvest, *DAMS**	36.9±0.14	33.0±0.25	28.6±0.24	27.2±0.14	27.4±0.1
Number of fruiting bodies, *pcs*	27.6±0.14	35.5±0.14	43.8±0.14	49.5±0.14	38.1±0.14
Number of fruit bunches, *pcs*	5.4±0.14	8.2±0.2	10.7±0.5	12.3±0.14	9.4±0.34
Weight of bunches, (g/bag^{-1})	92.8±0.25	84.6±0.25	75.6±0.25	63.7±0.2	88.2±0.19
Fruit body weight, (g/bag^{-1})	19.4±0.42	17.3±0.14	15.7±0.3	13.3±0.25	11.3±0.14
Biological productivity, *%*	438.9±3.16	580.0±7.74	795.2±3.98	806.0±5.83	921.6±4.2
Economic productivity, *%*	403.8±3.9	523.8±6.6	727.0±5.8	767.2±6.7	895.2±4.4
Biological effectiveness, *%*	61.4±1.2	68.5±1.1	72.8±0.29	106.7±0.61	105.8±1.0

**DAMS* – days after mycelium sowing
Values are mean ± standard deviation

Increasing the proportion of CS in the substrate formulas shows a slowdown in the total colonisation periods from 14.2 days on the control substrate sample without CS (C6) to 20.3 days on C2 with the highest CS proportion of 80 %. In addition, on the substrate that did not contain wheat straw (C1), complete colonisation did not occur.

A delay in the stages of complete colonisation of the substrate by mycelium was recorded compared to the control C6 by 6.2 days in C2, 5.0 days in C3, and 0.9 days in C4. At the same time, the addition of coffee sludge in the ratio of 50/50 practically did not affect the period of total colonisation of the substrate by mycelium, as well as the period of pin head initiation. A slight delay in initiation was observed in the C4 substrate formula, whereas in the other mixed substrates, a greater slowdown in this period was observed with increasing coffee grounds content.

The number of bunches and fruit bodies was higher on samples C5 and C4 compared to the control sample without coffee sludge, but this indicator decreased on samples C2 and C3. The highest bunch weight and fruit body weight were recorded in the substrates with an increased amount of coffee sludge. But on these substrates, there was a decrease in biological yield, economic yield and biological efficiency by almost half compared to the control substrate.

The inhibition of mycelial colonisation and the decrease in biological yield in substrates with a high content of coffee sludge can be explained by the increase in the content of lignin, caffeine and other inhibitors, including phenolic acids in substrates, which interfere with the fungal enzyme at an early stage of colonisation [15; 32]. And also low C/N ratio, which affects the mycelial growth rate [15].

It is known that plant extracts containing biologically active substances, such as bioflavonoids, protein, lipid and mineral substances, are known to be mycelial growth activators [33]. The effect of alfalfa extract on the growth and production of mushrooms was studied (Table 4).

Table 4

Effect of substrate on the main indicators of mushroom growth and production

Main characteristics	C8	C9	C10	C11	C12
Total colonization periods, *DAMS**	18.3±0.3	15.2±0.1	12.3±0.19	11.5±0.14	11.2±0.1
Pin head initiation after full colonization, *DAMS**	23.3±0.24	20.2±0.3	16.9±0.2	15.3±0.3	14.1±0.3
First wave of harvest, *DAMS**	28.4±0.28	21.9±0.4	20.4±0.3	19.2±0.24	19.5±0.4
Second wave of harvest, *DAMS**	32.4±0.4	26.6±0.4	25.5±0.14	24.2±0.19	24.1±0.1
Number of fruiting bodies, *pcs*	32.3±0.2	40.5±0.4	47.9±0.41	53.6±0.28	41.0±0.19
Number of fruit bunches, *pcs*	7.3±0.33	10.4±0.24	14.4±0.23	15.3±0.19	13.2±0.2
Weight of bunches, (g/bag^{-1})	102.5±0.4	94.4±0.25	82.1±0.7	68.3±0.3	92.8±0.29
Fruit body weight, (g/bag^{-1})	23.1±0.3	21.5±0.41	18.7±0.3	16.4±0.4	14.3±0.14
Biological productivity, *%*	458.0±0.9	599.0±0.6	806.3±4.7	827.4±1.9	939.2±5.36
Economic productivity, *%*	455.4±5.2	553.7±10.3	768.8±3.3	784.7±10.3	936.2±6.0
Biological effectiveness, *%*	86.8±0.64	84.0±2.18	94.3±0.9	138.4±0.6	135.2±0.52

**DAMS* – days after mycelium sowing
Values are mean ± standard deviation

In general, alfalfa extract improves all indicators of mushroom growth and production. The increase in the proportion of CS in the substrate formulas treated with alfalfa extract shows a slowdown in the total colonisation periods from 11.2 days on the control substrate sample without CS (C6) to 18.3 days on C8 with the highest CS proportion of 80 %. As in the case of the substrate untreated with alfalfa extract, complete colonisation did not occur on the substrate without wheat straw (C7). The total colonisation periods when treating substrates with alfalfa extract decreased within 2–3.1 days, with this indicator being better for substrates C10-C12 with a mass concentration of CS of 50–70 %.

A delay in the stages of complete colonisation of the substrate by mycelium was recorded compared to the control C12 by 7.1 days in C8, 3.1 days in C9, 1.1 days in C10, which reflects a similar trend for the untreated substrate. The activation of the stages of complete colonisation of the substrate by mycelium after treatment with alfalfa extract varied in the range of 3.2–5.1 days, or 14.96–26.56 %. At the same time, the addition of coffee sludge in a ratio of 50/50 had practically no effect on the period of total colonisation of the substrate by mycelium, as well as the period of pin head initiation. A slight delay in initiation was observed in the C10 and C11 substrate formulas, while in other mixed substrates, with an increase in the content of coffee grounds, a greater slowdown in this period was observed. Thus, compared to the control sample C12, this delay was 9.2 days for C8 and 6.1 days for C9.

The number of bunches and fruit bodies was higher on samples C10 and C11 compared to the control sample without the addition of coffee sludge, but this indicator decreased on samples C8 and C9. The highest bunch weight and fruit body weight were recorded in the substrates with an increased amount of coffee sludge. But on these substrates, there was a decrease in biological yield, economic yield and biological efficiency by almost half compared to the control substrate.

The activation of mycelial colonisation and the increase in biological yield in all substrates treated with alfalfa extract can be explained by the presence of substances of various nature in the extract, namely bioflavonoids, proteins, mineral and lipid substances, as well as an increase in the C/N ratio, which contributes to the growth rate of mycelium [34; 35].

Therefore, only substrates made on the basis of 100 % CS are not suitable for the cultivation of mushrooms. On substrates with the formulas of CS: wheat straw 50 : 50, 60 : 40, 70 : 30, 80 : 20, a full-fledged process of cultivation of Pleurotus ostreatus mushrooms takes place. The treatment of substrates with alfalfa extract accelerated the colonisation period by 2–3.1 days, the activation of the stages of complete colonisation of the substrate by mycelium after treatment with alfalfa extract was 14.96–26.56 %. The high proportion of CS in the substrate leads to a decrease in biological efficiency and yield, while the treatment of substrates with alfalfa extract increases biological yield, economic yield and biological efficiency. The mushrooms obtained in all treatment options are suitable for sale and this technology can be used as a way to safe ecological disposal of coffee waste.

All further studies were conducted using substrates treated with alfalfa extract.

The approximate chemical composition of Pleurotus ostreatus mushrooms cultivated on substrates with different concentrations of coffee sludge is shown in Table 5. Chemical composition of Pleurotus ostreatus fruits grown on different substrates

Table 5

Chemical composition of Pleurotus ostreatus fruits grown on different substrates

MOAS*	Total protein content, *%*	Fiber, *%*	Total carbohydrates, *%*	Fats, *%*	Total soluble sugars, *%*
C8	3.16±0.028	3,93±0.05	5,76±0.04	0.28±0.029	0.017±0.002
C9	3.10±0.099	3.95±0.052	5.85±0.053	0.26±0.03	0.026±0.005
C10	2.93±0.054	3.63±0.063	6.42±0.058	0.19±0.014	0.03±0.0009
C11	2.88±0.04	3.47±0.07	7.69±0.019	0.16±0.005	0.037±0.007
C12	3.25±0.36	4.12±0.02	6.46±0.029	0.18±0.009	0.015±0.001

*MOAS – Mushrooms of appropriate substrates
Values are mean ± standard deviation

The total protein content of mushrooms grown on substrates with the addition of coffee sludge was slightly lower compared to the control substrate without the addition of coffee sludge. Thus, in mushrooms cultivated on substrates C8 and C9, the difference with the protein content in mushrooms cultivated on the control sample C12 was 0.09 % and 0.15 %, respectively, and in mushrooms on substrates C10 and C11 – 0.32 % and 0.37 %, respectively.

Although the fungi cultivated on substrates with the addition of CS had a high fibre content, the highest amount of fibre was recorded in the fungi cultivated on the control sample of the substrate without the addition of CS (C12). In mushrooms on substrates C8 and C9, the total crude fibre content was almost the same and the difference with the mushrooms on the control sample was about 0.2 %, and in mushrooms on substrates C10 and C11 – 0.5 % and 0.7 %, respectively.

The total carbohydrate content of Pleurotus ostreatus ranged from 5.76 % to 7.69 %. Their highest content was presented in mushrooms cultivated on substrate C11, and the lowest - on C8. The mushrooms cultivated on the control sample C12 had a 1.2 % lower carbohydrate content compared to the mushrooms cultivated on the C11 substrate, but 0.6 % and 0.7 % higher compared to the mushrooms on C9 and C8, respectively. The difference between the carbohydrate content of mushrooms cultivated on substrate C10 and the control sample was rather insignificant and amounted to 0.04 %.

In mushrooms cultivated on substrates C8, C9, C10, the fat content was higher compared to mushrooms grown on C12 by 0.1 %, 0.08 % and 0.01%, respectively. However, the fat content of mushrooms on C11 was lower by 0.02 % compared to the control sample.

The richest in total soluble sugars were Pleurotus ostreatus mushrooms cultivated on substrates C9, C10 and C11. Mushrooms cultivated on the control sample had the lowest sugar content.

The ability of fungi to bioaccumulate minerals is influenced by various environmental factors: the chemical composition of the substrate, fungal species, morphological part of the fruiting body, etc [1]. The ability of mushrooms to accumulate a significant proportion of micro and macro elements that have a positive effect on the human body is of great interest not only in the food but also in the medical industry [34; 35]. The mineral composition of mushrooms grown on different substrates is shown in Table 6.

Table 6

Total content of micro and macro elements in mushrooms Pleurotus ostreatus (% dry weight)

MOAS*	Micro and macro elements					
	Ca	K	Mn	Fe	Na	Mg
C8	0.0029±0.0001	0.37±0.014	0.00011±0.00001	0.0011±0.0002	0,004±0.0002	0.014±0.008
C9	0.0027±0.00009	0.34±0.0009	0.00009±0.00	0.0014±0.00000	0.006±0.00009	0.014±0.00001
C10	0.0025±0.00	0.31±0.001	0.00008±0.0000014	0.0018±0.00000	0.008±0.0002	0.014±0.000014
C11	0.0016±0.00009	0.28±0.01	0.00006±0.000002	0.0018±0.00000	0.008±0.0002	0.013±0.00009
C12	0.0031±0.00009	0.29±0.01	0.0001±0.00002	0.0017±0.00000	0.048±0.0005	0.013±0.00001

*MOAS – Mushrooms of appropriate substrates
Values are mean ± standard deviation

Pleurotus ostreatus mushrooms cultivated on different substrates with different contents of CS showed a rather diverse content of micro and macro elements. High potassium content was typical for mushrooms grown on all types of substrates. Table 6 shows that the increase in the proportion of coffee sludge in the substrate formulas was positively correlated with the potassium content of mushrooms grown on these substrates.

The manganese content in Pleurotus ostreatus mushrooms was quite high. The increase of coffee sludge as a component of the substrate did not show a significant effect on the change in its amount in mushrooms grown on these substrates. Thus, the amount of manganese in mushrooms cultivated on C8, C9, C10 and C12 was almost the same and 0.002 % higher than in mushrooms grown on C11. In mushrooms grown on substrates C10 and C11, the level of iron was almost the same and slightly exceeded the level of iron in mushrooms grown on the control substrate C12. However, with further increase of the share of CS in the substrate formulas, the iron level began to decrease. Already in mushrooms grown on substrate C8 the amount of iron was lower than in mushrooms grown on C12. Also, sodium, calcium and traces of magnesium were determined in the composition of all mushrooms.

Studies of the fatty acid composition of oyster mushroom Pleurotus ostreatus fruits revealed the content of polyunsaturated (linoleic and linolenic), monounsaturated (oleic, palmitoleic) and saturated (palmitic and stearic) fatty acids. The approximate fatty acid composition of oyster mushrooms Pleurotus ostreatus is shown in Table 7.

Table 7

Fatty acid composition of oyster mushrooms Pleurotus ostreatus

MOAS*	PFA*, %		MFA*, %			SFA*, %	
	linoleic	linolenic	palmitoleic	oleic	arachidic	palmitic	stearic
C8	82.99±0.41	0.39±0.005	0.77±0.009	5.03±0.01	–	9.64±0.085	0,72±0.0025
C9	82.73±0.08	0.36±0.005	0.77±0.014	5.82±0.016	–	9.84±0.12	0.76±0.07
C10	81.48±0.4	–	0.41±0.009	6.54±0.028	–	10.18±0.025	0.81±0.009
C11	80.57±0.2	–	0.25±0.01	7.89±0.02	–	10.12±0.03	0.77±0.02
C12	80.43±0.09	0.23±0.002	0.09±0.002	9.27±0.001	–	9.27±0.001	0.74±0.002

*MOAS – Mushrooms of appropriate substrates; PFA – polyunsaturated fatty acids; MFA – monounsaturated fatty acids; SFA – saturated fatty acids
Values are mean ± standard deviation

The analysis of the obtained profile showed that polyunsaturated fatty acids were the main fatty acids of Pleurotus ostreatus mushrooms cultivated on different substrates. Their share was more than 84 %. This proves the positive effect of eating such mushrooms on the human body, as polyunsaturated fatty acids reduce the risk of developing diabetes and reduce the incidence of coronary heart disease.

The main share of polyunsaturated fatty acids was linoleic acid (about 80–83 %). A slight increase in the content of this acid in mushrooms was observed, with an increase in the proportion of PUFAs in the substrates on which they were cultivated. The content of linolenic acid was quite low (0.2–0.4 %), and it was not detected on C11 and C10. Mushrooms grown on C8 and C9 showed the richest composition of polyunsaturated fatty acids.

Saturated fatty acids can have a negative impact on the human body, as they have a low density and can lead to diabetes. The proportion of such acids in mushrooms reached about 12 %, so their moderate consumption does not have a negative impact on the body. Arachidic acid was not detected in the mushrooms, although it was present in the substrates on which they were cultivated. This indicates that it is completely converted by Pleurotus ostreatus into other components. The main share of saturated fatty acids was palmitic acid – about 10–11 %, depending on the composition of the substrate. Stearic acid was detected in an amount not exceeding 1 %.

The implementation of the principle of waste-free production is an important factor in increasing the efficiency of agricultural production, so all by-products of the processing industry, agriculture and restaurant business that have potential nutritional value should be used in the feeding of farm animals. The results of the study of waste substrates are shown in Table 8. Chemical composition of waste substrates with different CS content.

Table 8

Chemical composition of waste substrates with different SCG content

Substrates	Total protein content,%	Fiber,%	Total carbohydrates,%	Fats,%	Total soluble sugars,%
C8	13.90±0.24	27.67±0.02	32.41±0.06	4.97±0.04	0.12±0.005
C9	13.10±0.09	28.7±0.19	33.10±0.43	4.63±0.03	0.13±0.00
C10	12.9±0.14	31.9±0.01	33.48±0.09	4.11±0.02	0.13±0.009
C11	11.05±0.009	36.71±0.1	35.11±0.03	3.75±0.07	0.14±0.02
C12	5.70±0.2	40.54±0.07	39.96±0.84	0.68±0.005	1.58±0.09

Values are mean ± standard deviation

All proteins are of fundamental, primary importance in the construction of the body and in the vital functions of living organisms. Increasing the proportion of protein in the diet of cattle plays a positive role in their overall development. Table 8 shows that the total protein content increased in all waste substrates containing CS in different proportions compared to the control substrate C12. The highest proportion of protein was observed in substrates C8 and C9 – approximately 14 % and 13 %, respectively. The results of the study demonstrated a direct dependence of the increase in the proportion of protein in the waste substrates on the proportion of coffee sludge in the formulas of these substrates.

Insufficient levels of fibre in animal nutrition can lead to impaired digestive function and ruminative activity. At the same time, excessive fibre content reduces the digestibility of nutrients in the diet. The more fibre a feed contains, the lower its nutritional value. As plants grow and age, their fibre content increases. Straw has the highest fibre content (27–42 %) compared to other types of livestock feed. Adding a share of CS to the substrate led to a decrease in the amount of fibre in the waste substrate, which is an indicator of the possibility of using the waste substrate as a feed additive. Thus, the lowest percentage of fibre was recorded in sample C8, and the highest in the control sample of substrate C12 without the addition of coffee sludge.

The total carbohydrate content, on the contrary, was the highest in the substrates without the addition of CS and gradually decreased with an increase in its share in the substrates. The carbohydrate content of samples C9 and C10 was almost the same with a small difference of 0.38 %. Although mushrooms grown on substrate C11 had a higher carbohydrate content than mushrooms grown on a substrate without coffee sludge, the spent substrate C11 itself had a lower carbohydrate content than C12.

Fat is a source of energy. A lack of it in the diet of animals leads to vitamin deficiency, skin diseases and impaired reproductive capacity. According to the study, the addition of CS to substrates positively correlated with the fat content of the spent substrates. Thus, the fat content in the C8 substrate was almost 7.3 times higher than in the C12 control sample.

The control substrate C12 was the richest in total soluble sugars, including fructose, glucose and sucrose, compared to the other substrates. The total amount of soluble sugars in samples C9 and C10 was almost the same, but 12 times less than in the control substrate C12.

Minerals are essential for building bone tissue and for many physiologically important substances, such as enzymes. Minerals such as calcium, potassium, iron, sodium, and magnesium are considered essential for life. An analysis of the mineral composition of waste substrates is shown in Table 9.

Table 9

Mineral composition of waste substrates with different SCG content (% dry weight)

Substrates	Micro and macro elements					
	Ca	K	Mn	Fe	Na	Mg
C8	0.35±0.0023	0.07±0.0014	0.0021±0.00003	0.031±0.0004	0.14±0.005	0.13±0.004
C9	0.37±0.004	0.01±0.005	0.0023±0.00002	0.025±0.0004	0.14±0.002	0.11±0.002
C10	0.52±0.002	0.12±0.003	0.0026±0.00003	0.018±0.0004	0.13±0.002	0.11±0.003
C11	0.55±0.002	0.17±0.002	0.0031±0.00004	0.017±0.0002	0.13±0.002	0.11±0.009
C12	0.45±0.003	1.39±0.003	0.09±0.002	0.013±0.0002	0.06±0.003	0.06±0.002

Values are mean ± standard deviation

The calcium content of the substrate formulas increased initially with the addition of CSA. Thus, substrates C11 and C10 had a slightly higher level of calcium (0.55 % and 0.52 %, respectively) compared to the control sample of substrate C12 (0.45 %). However, with a further increase in the proportion of CS to 70–80 %, the calcium content began to decrease (C9 – 0.37 %; C8 – 0.35 %). The amount of iron, sodium, and magnesium increased with the addition of CS in the spent substrates. Thus, in the C8 substrate, the amount of iron was 0.031 %, while in the control substrate C12 it was 0.013 %. The manganese content for substrates C8 and C9 was 0.21 % and 0.23 %, respectively. And on the spent C12 substrate, it was only 0.09 %. The amount of sodium was almost the same on substrates C8 and C9 and on C8 and C10 and C11 (0.14 % and 0.13 %, respectively). While on the control sample without CG (C12) it was only 0.06 %. With an increase in the proportion of CS in different substrate formulas, the indicators of potassium and manganese decreased.

The content of fatty acids in the spent substrates with different proportions of CS is more than 50 % as shown in Table 10.

Table 10

Fatty acid composition of waste substrates

Substrates	PFA*, %		MFA*, %		SFA*, %		
	linoleic	linolenic	palmitoleic	oleic	arachidic	palmitic	stearic
C8	50.93±0.35	2.35±0.04	–	13.1±0.2	1.3±0.01	18.01±0.01	5,52±0.004
C9	51.63±0.09	2.95±0.004	–	13.58±0.01	1.95±0.005	28.9±0.03	6.55±0.05
C10	47.21±0.05	3.55±0.08	–	14.39±0.08	2.07±0.02	25.65±0.09	7.94±0.02
C11	42.45±0.1	4.02±0.009	–	14.53±0.14	2.15±0.004	27.06±0.005	8.62±0.005
C12	22.22±0.1	8.16±0.03	1.6±0.03	11.28±0.005	–	54.4±0.03	5.87±0.02

*PFA – polyunsaturated fatty acids; MFA – monounsaturated fatty acids; SFA – saturated fatty acids
Values are mean ± standard deviation

Preliminary analysis of coffee sludge for fatty acids showed that it contains 42.9 % linoleic acid, 3.9 % linolenic acid, 10.4 % oleic acid, 0.8 % arachidic acid and 9.7 % stearic acid.

In the control substrate C12 without the addition of CS, the content of linoleic acid was the lowest - approximately 22 %, and then, with the addition of CS, its share in the corresponding waste substrates increased. Thus, in substrates C11, C10, and C9, the proportion of linoleic acid was already approximately 42.5 %, 47 %, and 51.6 %, respectively. However, on the spent substrate with the highest content of coffee sludge C8, its amount decreased compared to substrate C9 and amounted to almost 51 %. Although no linolenic acid was detected in the mushrooms grown on substrates C10 and C11, the amount of linolenic acid in these waste substrates was almost 4 %, which is 4.14 % less than in the control substrate C12. Further, with an increase in the proportion of CS in the substrates, its content decreased. As for palmitoleic acid, it was not detected in any waste substrate with any CS content. Substrates with CS content were richer in oleic acid compared to the control substrate C12. The highest content of arachidic acid was in substrates C11 and C10 (2.15 % and 2 %, respectively), in substrates with a higher content of coffee sludge C9 and C8 its amount was lower (1.95 % and 1.3 %, respectively), and in the control substrate without CS it was absent at all. The highest level of palmitic acid was recorded in the control substrate C12, which did not contain CS at all. The presence of palmitic and palmitoleic acids in the spent substrates can be explained by the content of wheat straw in the substrate formulas, since the CS did not contain both fatty acids. Substrates C11 and C10 had the highest stearic acid content (7.9 % and 8.6 %, respectively), and substrates C8 and C12 had the lowest stearic acid content (5.5 % and 5.9 %), respectively.

To test the ability of Pleurotus ostreatus to degrade caffeine and phenol in substrates containing more than 50 % CS, samples were taken before and after the cultivation process. The data obtained are presented in Table 11.

Table 11

Changes in total phenols and caffeine content (mg/100 g dry weight) in mixed substrates

Substrates	Phenols			Caffeine		
	Fresh substrate	Waste substrates	Difference, (%)	Fresh substrate	Waste substrates	Difference, (%)
C12	224.0±1.9	200.6±0.24	10.4	–	–	–
C11	263.7±0.2	197.7±1.9	25	458.15±0.14	189.4±1.3	57.8
C10	353.6±0.5	208.5±0.8	41.4	532.01±0.6	163.2±0.5	63.9
C9	489.4±1.07	235.3±3.8	51.9	584.80±0.48	132.96±0.9	77.3
C8	548.2±0.99	249.3±0.99	54.5	654.4±0.72	127.56±0.2	80.5

Values are mean ± standard deviation

All substrates, regardless of the proportion of CS in them, showed a decrease in phenol and caffeine content compared to their initial concentrations before cultivation. The highest percentage of phenol reduction in the spent substrate was observed in substrates with the highest CS content – C8 and C9 (54.5 % and 51.9 %, respectively). The highest percentage of caffeine reduction was observed in substrates C8, C9 and C10 (80.5 %, 77.3 % and 69.3 %, respectively).

Fungi of the genus Pleurotus are the most efficient lignin-degrading organisms. They degrade lignin with enzymes collectively referred to as ligninases. Ligninases can be classified as phenol oxidases (laccases), heme oxidases (lignin peroxidases, manganese peroxidases, and universal peroxidases) [36]. Previous studies have shown that cultivation of Pleurotus on substrates containing CS shows increased concentrations of laccase and MnP (responsible for lignin degradation and phenolic detoxification during mycelial propagation) [23; 37]. A relatively high concentration of phenolic content in the CS provokes favourable conditions for extracellular laccase biosynthesis due to increased laccase gene expression [37]. Thus, a decrease in the phenolic content of the substrate after cultivation is associated with greater enzymatic detoxification of coffee waste [23; 37].

Conclusions

Based on the results of this study, it can be concluded that the mycelium of the fungi of the genus Pleurotus ostreatus can completely colonise substrates containing a high proportion of CS. Although the substrates with a higher proportion of coffee grounds in their composition give slightly lower indicators of mushroom growth and production (total colonisation period, biological yield and efficiency, etc.), the resulting product is of good quality with a fairly rich and diverse composition and high organoleptic characteristics. The cultivation of mushrooms was not possible only on the substrate with a proportion of 100 % CS.

The treatment of substrates with alfalfa extract accelerated the colonisation period by 2–3.1 days, and the activation of the stages of complete colonisation of the substrate by mycelium after treatment with alfalfa extract was 14.96–26.56 %. The high proportion of CS in the substrate leads to a decrease in biological efficiency and yield, while the treatment of substrates with alfalfa extract increases biological yield, economic yield and biological efficiency.

The study of the spent substrates shows that they are quite nutritious. In addition to the fact that all substrates in the formulation of which CS was used contained a significant amount of proteins, fats, minerals necessary for the daily diet of livestock, they also showed a decrease in the content of phenols and caffeine. This confirms the possibility of their use as a daily protein supplement to animal feed.

The improvement of many nutritional aspects of P. ostreatus grown on wheat straw/CS substrate and the possibility of using the spent substrate as an animal feed additive indicates that CS is a nutritious potential supplement to commercial wheat straw.

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