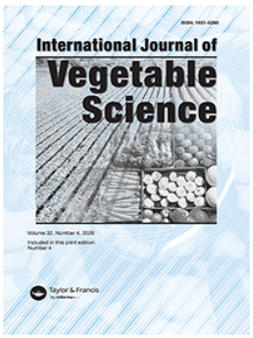

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Effects of phenolic-rich agro-industrial by-product supplementation on yield, nutritional content and antioxidant capacity of *Pleurotus djamor*

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ABSTRACT

Wheat bran is widely used as a supplement in *Pleurotus* cultivation, but it can be costly and not always available, prompting nutrient and bioactive-rich alternatives. In this study, four phenolic-rich residues, used tea leaves, olive press cake, grape pomace, and green walnut hulls, were used as supplements to wheat straw in the cultivation of *Pleurotus djamor,* and the effects of the cultivation substrates on yield, biological efficiency, nutritional composition, total phenolic content, antioxidant activity, and Fourier-transform infrared spectroscopy-derived functional group profiles were determined. Adding 20% used tea leaves increased total yield by 34% to 213.0 g/kg; olive press cake accelerated mycelial growth (15 d) and shortened harvest time (23.6 d). Mushrooms contained 15.17–26.25 g/100 g protein and 72.17–74.81 g/100 g carbohydrate and 1.24–1.56 g/100 g fat on a dry-weight basis. Total phenolic content correlated positively with antioxidant activity, and the control showed the highest values. FTIR spectra indicated substrate-dependent shifts. Overall, phenolic-rich supplements can improve performance and shape composition, but they do not consistently raise fruiting body total phenolic content or antioxidant levels; used tea leaves appear the most promising substrate for *P. djamor* cultivation.

KEYWORDS

Pink oyster mushroom; used tea leaves; olive press cake; grape pomace; green walnut hulls

Introduction

Edible mushrooms have come to the forefront among functional foods in recent years. The reason for this is not only their low calorie and fat content, but also the fact that they contain significant amounts of protein and provide a wide variety of vitamins and minerals (Abedini et al. 2022). In addition, many species are known to synthesize polysaccharides and other metabolites. These compounds have been shown to exhibit antioxidant properties and to regulate immune functions at both laboratory and clinical studies. On the other hand, mushroom cultivation is gradually gaining biotechnological value.

In regions where agriculture dominates, plant by-products are often used as animal feed, soil amendments, or for biofuel. Many of these residues are also

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disposed of through burning or by mixing them back into the soil. An alternative approach is to employ such materials in mushroom cultivation, which offers clear advantages for sustainable production. Within this framework, the genus *Pleurotus* has gained attention because it can grow on a wide range of substrates while maintaining high biological efficiency. Among its species, *Pleurotus djamor*, commonly known as the pink oyster mushroom, is notable for its rapid substrate colonization, adaptability, and high yield under diverse environmental conditions (İnci et al. 2024).

The composition of the substrate is an important factor that directly influences the success of mushroom cultivation (Hoa et al. 2015). It affects not only mycelial growth and yield but also the nutritional composition of the fruiting bodies (Diamantopoulou et al. 2023). Substrate physicochemical conditions, particularly nitrogen availability and the C:N balance, significantly influence colonization rate and yield outcomes in *Pleurotus* (Silva et al. 2024; Subedi et al. 2023; Wei et al. 2024). Because these performance outputs also determine the economic feasibility of production, biological efficiency is widely used as a practical indicator of feasibility in cultivation (Ahmed et al. 2024; Ritota and Manzi, 2019; Solórzano et al. 2025).

Mushrooms, through their natural bioaccumulation ability, can absorb nutrients and minerals from the growth medium and transfer them into their fruiting structures. Research has shown that enriching substrates with trace elements such as zinc, iron, selenium, and magnesium can increase biomass production and improve the functional qualities of cultivated species (Słyszyk et al. 2024). In addition, mushrooms have been reported to possess a notable level of antioxidant capacity. This feature offers a safer alternative to synthetic antioxidants (e.g., BHA, BHT, TBHQ), which have been associated with toxic and carcinogenic risks (Chandra et al. 2020).

Agro-industrial by-products such as used tea leaves (UTL), olive press cake (OPC), grape pomace (GP), and green walnut hulls (GWH) are organic materials rich in phenolic compounds. However, their high phenolic content can cause problems such as phytotoxicity and the inhibition of microbial growth, which in turn limit their biodegradability and bioavailability (Dey et al. 2021). When these residues are disposed of without control, they contribute to rising waste management costs, soil and water contamination, and greenhouse gas emissions from burning practices. At the same time, their lignocellulose-rich composition makes them a promising resource for mushroom cultivation. Through the bioconversion capacity of fungi, low-value organic wastes can be transformed into high-value functional foods. In addition, the use of phenolic-rich agricultural residues may help enrich mushrooms with antioxidant compounds, thereby enhancing their nutritional and pharmacological potential.

FTIR spectroscopy is increasingly used as a complementary tool to track compositional fingerprints of mushroom biomass and interpret cultivation-

induced chemical changes (Koutrotsios et al. 2019; Synytsya et al. 2023), which is particularly relevant for phenolic-rich supplementation where chemical shifts may occur despite variable yield responses.

This research aims to assess the effects of agro-industrial by-products rich in phenolics, as sustainable alternatives to wheat bran, a conventional substrate supplement, on the growth performance, yield, and biochemical composition of *P. djamor*. Although these by-products are abundantly available, their applications in mushroom cultivation have not been sufficiently investigated. In particular, their influence on the accumulation of bioactive compounds remains poorly understood. The purpose of the study can be summarized as follows: (I) to determine the dose-dependent and material-specific effects of phenolic-rich substrate supplementation on the biological efficiency and total yield of *P. djamor* fruiting bodies; (II) to examine the effects of such supplementation on the nutritional profile, total phenolic content (TPC), and antioxidant properties of *P. djamor*; and (III) to evaluate the impact of alternative substrates on the chemical structure and functional group variations of *P. djamor* as determined by Fourier-transform infrared (FTIR) spectroscopy.

Materials and methods

Materials

A pure culture of *P. djamor* was obtained from Homegreen Company (Netherlands) and maintained on potato dextrose agar (PDA) for the preparation of wheat grain spawn.

The cultivation substrates were sourced from different local producers: wheat straw and green walnut hulls were collected from farms in Kırşehir; OPC was supplied by an olive oil facility in Menemen, Izmir; used tea leaves were provided by a cafeteria near Kırşehir Ahi Evran University; and grape pomace came from a winery in Nevşehir. All experiments were carried out in 2023 at the Mushroom Research Laboratory, Kırşehir Ahi Evran University, Türkiye.

Spawn production

Wheat grains were moistened to a final water content of 55–60% (w/w) and sterilized in polypropylene bags at 121°C for 90 min. After the grains had cooled, they were inoculated with *P. djamor* mycelium maintained on PDA medium. They were then incubated in the dark at 25°C, and were gently shaken three times at 3-day intervals until mycelial growth was complete.

Table 1. Formulations of growing media and ratios of basal substrate and supplement material used in the study.

Treatment	Basal substrate	Basal Substrate Ratio (w/w)	Supplement material	Supplement material (w/w)
Control	Wheat straw	80%	Wheat bran	20%
WS9:UTL1	Wheat straw	90%	Used tea leaves	10%
WS8:UTL2	Wheat straw	80%	Used tea leaves	20%
WS9:OPC1	Wheat straw	90%	Olive press cake	10%
WS8:OPC2	Wheat straw	80%	Olive press cake	20%
WS9:GP1	Wheat straw	90%	Grape pomace	10%
WS8:GP2	Wheat straw	80%	Grape pomace	20%
WS9:GWH1	Wheat straw	90%	Green walnut husks	10%
WS8:GWH2	Wheat straw	80%	Green walnut husks	20%

Preparation of mushroom growing media

The control substrate consisted of 80% wheat straw and 20% wheat bran on a dry weight basis (Table 1). The other cultivation substrate formulations were prepared by supplementing wheat straw with 10% and 20% of OPC, GWH, GP and UTL. During substrate preparation, the materials were immersed in water for 24 h to allow proper hydration, after which excess water was thoroughly drained. The hydrated substrates were then mixed thoroughly in the specified proportions and packed into heat-resistant polypropylene bags (29 × 45 cm, 1 kg each). The bags were sealed with cotton plugs and secured with rubber bands. Sterilization was carried out in an autoclave at 121°C (1.2 atm) for 90 min. After sterilization, spawn was added to each bag at a rate of 3% (w/w) and incubated at 25 ± 2°C with 85% relative humidity. During incubation, no fresh air exchange was provided in the incubation room; however, each polyethylene bag had micro-perforations that allowed passive gas exchange (oxygen supply and carbon dioxide release) during mycelial growth. Once the incubation phase was completed, the cotton plugs were taken out and the bags were transferred to the fruiting chamber. At this stage, light intensity was maintained at 500 lux for 12 h daily using fluorescent lamps. Carbon dioxide levels were kept below 1000 ppm through controlled air exchange. Relative humidity was adjusted to 80–90%, and the temperature was maintained at 25 ± 2°C.

The experiment was designed according to a one-factor Completely Randomized Design with ten replicates (bags) for each substrate formulation. The recorded data included mycelial growth period, time to pinhead formation, time to first harvest, total mushroom yield (g), and biological efficiency (BE), which was calculated using Equation 1

$$BE(\%) = \frac{\text{weight of fresh mushroom per bag}}{(\text{weight of dry growing media})} X100 \quad (1)$$

Chemical analysis of growing media

Chemical composition analyses of the growing media were conducted following standard AOAC methods (AOAC International, 2021). Moisture content was measured by weighing samples before and after the samples were dried at 105°C until constant weight was obtained. The Kjeldahl method was employed to determine the N content. Ash content was assessed by incineration at 600°C for 2 h. C (%) was estimated through Equation 2:

$$C(\%) = (100 - Ash\%)\, X\, 0.58 \tag{2}$$

The C/N ratio was then computed based on obtained C and *N* values. All analyses were conducted in triplicate.

Nutritional composition of fruiting body

Drying of fruiting bodies was carried out at 40°C until constant weight was obtained, finely ground, and sieved prior to analysis. Moisture, ash, N, and crude fat contents were determined using AOAC International (2021) protocols.

Protein content was calculated as N (%) × 4.38. Crude fat was extracted using Soxhlet extraction with hexane as a solvent. Total carbohydrate and energy values were computed using Eqs S3 and 4, respectively:

$$Carbonhydrate\,(g/100g) = 100 - (moisture + ash + crudefat + protein) \tag{3}$$

(Heleno et al. 2009)

$$Energy\,(kcal/100g) = (4 \times protein + carbohydrate) + (9 \times crudefat) \tag{4}$$

(Heleno et al. 2009)

Evaluation of total phenolic content and antioxidant activity

The extraction of dried and ground *P. djamor* samples was performed with 99% ethanol (1:30 w/v) for 72 h at 130 rpm on an orbital shaker. The resulting extracts were passed through Whatman No. 1 filter paper, concentrated under reduced pressure with a rotary evaporator at 40°C, and kept at 4°C until further analysis (Atila et al. 2024).

TPC was determined by the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Measurements of absorbance were performed at 725 nm using a spectrophotometer (Shimadzu UV Mini 1240, Germany). Gallic acid served as the standard compound, and the results were expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g dw).

Antioxidant capacity was assessed via two methods: ABTS radical cations ($ABTS^{\bullet+}$) assay was performed following Hasbal et al. (2015), where ABTS was reacted with potassium persulfate and the mixture incubated in the dark for 12–16 h. The resulting solution was diluted with ethanol to adjust the absorbance to 0.70 ± 0.02 at 734 nm. Subsequently, 100 µL of each extract was incorporated into the reaction mixture, with absorbance measured after 6 min. The quantification of antioxidant activity was carried out with a Trolox calibration curve, with values expressed as TEAC (µmol TE/g DW).

Ferric reducing antioxidant power (FRAP) were assessed following the method of Benzie and Strain (1996). For extraction, 1 g of dry fruiting bodies was homogenized with 10 mL of methanol using an IKA Ultra-Turrax homogenizer. The resulting homogenates were maintained in the dark at 4°C for 14–16 h and subsequently filtered through Whatman No. 4 filter paper. The supernatants were collected and stored at – 20°C until analysis. Absorbance was read at 593 nm on a spectrophotometer (Shimadzu UV Mini 1240, Germany). Trolox was used as the reference antioxidant standard, and results were expressed as µmol Trolox equivalents per gram of dry weight. All analyses were conducted in triplicate.

Fourier transform infrared (FTIR) spectroscopic analysis

ATR-FTIR spectroscopy was employed to characterize organic compounds and functional groups in mushroom structures. Spectra were recorded at room temperature with an IRTracer-100 FTIR spectrometer (Shimadzu, Japan) equipped with an ATR unit and a DLATGS detector. Each spectrum was obtained from 128 scans at a resolution of 4 cm^{-1} over the range of 4000–450 cm^{-1}.

Statistical analysis

Statistical analysis was performed with SPSS software (version 16). Data were expressed as mean ± standard deviation (SD). One-way ANOVA with Tukey's post-hoc test was used to evaluate statistical significance ($p \leq .05$).

Results

Proximate composition of the growing media

Statistically significant differences were observed among the tested growing media in terms of moisture content, pH, ash, C, N, and C:N ratio ($p < .001$, Table 2).

The lowest moisture content was recorded in the WS9:OPC1 medium, while the highest was found in WS9:GWH2. In the present study,

Table 2. Proximate analysis of growing media tested in the study.

Treatments	Moisture (%)	pH	Ash (%)	Carbon (%)	Nitrogen (%)	C/N ratio
Control	69.8 ± 0.43 a	6.57 ± 0.05 cd	10.5 ± 0.2 b	52.1 ± 0.12 d	1.13 ± 0.04 a	46.1 ± 1.6 e
WS9:UTL1	68.0 ± 0.16 b	6.40 ± 0.05 d	7.9 ± 0.2 e	53.6 ± 0.12 a	0.71 ± 0.02 bc	75.9 ± 2.6 cd
WS8:UTL2	69.7 ± 0.59 a	6.59 ± 0.09 cd	8.6 ± 0.2 de	53.1 ± 0.09 ab	0.78 ± 0.01 b	68.5 ± 0.6 d
WS9:OPC1	67.4 ± 0.66 bc	6.65 ± 0.08 c	9.1 ± 0.6 cd	52.8 ± 0.32 bc	0.55 ± 0.01 e	95.6 ± 1.8 a
WS8:OPC2	67.9 ± 0.65 b	6.64 ± 0.12 c	8.3 ± 0.5 de	53.3 ± 0.29 ab	0.59 ± 0.04 de	90.1 ± 6.4 ab
WS9:GP1	67.8 ± 0.51 b	6.46 ± 0.07 cd	8.4 ± 0.2 de	53.3 ± 0.10 ab	0.61 ± 0.04 de	87.6 ± 5.4 abc
WS8:GP2	66.0 ± 0.29 c	7.08 ± 0.03 b	8.4 ± 0.2 de	53.3 ± 0.13 ab	0.67 ± 0.05 cd	80.2 ± 5.8 bcd
WS9:GWH1	66.9 ± 0.54 bc	7.14 ± 0.03 ab	10.1 ± 0.2 bc	52.3 ± 0.09 cd	0.72 ± 0.03 bc	72.7 ± 3.5 d
WS8:GWH2	66.0 ± 0.45 c	7.33 ± 0.06 a	12.4 ± 0.6 a	50.9 ± 0.34 e	0.67 ± 0.00 cd	76.0 ± 0.7 cd
P value	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*

Mean values in the same column followed by the same letters are not significantly different by Tukey's test ($n = 3$) (WS: wheat straw; UTL: used tea leaves; OPC; olive pres cake; GP: grape pomace; GWH: green walnut hulls).

N content ranged from 0.55% to 1.13%, remaining below the recommended upper threshold. The ash content of the substrates ranged from 7.9% to 12.4%, with the highest values observed in WS:GWH2 and the control medium. On the other hand, C content ranged between 50.9% and 53.6%, with variations depending on the composition of the tested substrates. As N content increased, the C:N ratio decreased. The control medium had the highest N content and the lowest C:N ratio (46.1), while WS8:OPC1, which had the lowest N content, exhibited the highest C:N ratio (95.6).

Mycelial growth and fruiting time

Statistically significant differences were found among the growing media in terms of spawn run (colonization) duration, primordia formation, and time to first harvest ($p < .001$, Table 3).

All nine growing media showed good development during the incubation period. The spawn run period ranged from 15.0 to 25.3 d across the different growing media. On the other hand, growing media containing 20% GWH delayed growth by approximately 3 to 10 d compared to the control and WS8:OPC2 media. Interestingly, similar spawn run periods (17.0–17.7 d) were observed in WS:UTL1, WS9:OPC1, and WS9:GP1 media with 10% UTL, OPC, and GP supplementation. However, increasing the additive material concentration to 20% for UTL and GP slowed mycelial growth, extending colonization time to 22.6 and 22.4 d, respectively.

The time required for pinhead formation and first harvest ranged from 19.1 to 33.6 d and 23.6 d to 39.6 d, respectively. GWH delayed both mycelial growth and pinhead formation, with the longest times for pinhead formation and first harvest occurring in the WS8:GWH2 medium. Conversely, the addition of 20% OPC accelerated the appearance of basidiomata, with fruiting bodies forming 7 d earlier than in the control medium (19.1 d).

Table 3. Effect of growing media on cultivation cycle and yield of *Pleurotus djamor*.

Growing Media	Spawn run time (days)	Days to initial of primordia (days)	Days to first harvest (days)	Total Yield (g/kg)	Biological Efficiency (%)
Control	21.9 ± 0.64 b	26.1 ± 0.83 c	29.6 ± 0.49 d	158.6 ± 2.92 ef	52.9 ± 0.97 c
WS9:UTL1	17.6 ± 0.49 c	23.6 ± 0.90 d	33.4 ± 0.90 c	184.1 ± 5.54 c	57.5 ± 1.73 b
WS8:UTL2	22.6 ± 0.90 b	32.1 ± 0.64 b	36.7 ± 0.45 b	213.0 ± 7.42 a	71.0 ± 2.47 a
WS9:OPC1	17.0 ± 0.76 c	21.9 ± 0.64 e	26.0 ± 0.93 f	162.0 ± 5.07 ef	49.1 ± 1.54 d
WS8:OPC2	15.0 ± 0.93 d	19.1 ± 0.83 f	23.6 ± 0.90 g	164.8 ± 5.76 de	51.5 ± 1.80 cd
WS9:GP1	17.7 ± 0.70 c	22.9 ± 0.64 de	27.7 ± 0.45 e	173.4 ± 4.00 d	54.2 ± 1.25 c
WS8:GP2	22.4 ± 0.49 b	27.4 ± 0.90 c	32.9 ± 0.64 c	167.2 ± 3.34 de	52.3 ± 1.05 c
WS9:GWH1	21.6 ± 0.49 b	26.6 ± 0.49 c	30.7 ± 0.45 d	195.0 ± 7.29 b	59.1 ± 2.21 b
WS8:GWH2	25.3 ± 0.70 a	33.6 ± 0.49 a	39.6 ± 0.49 a	152.6 ± 4.32 f	44.9 ± 1.27 e
p value	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Mean values in the same column followed by the same letters are not significantly different by Tukey's test ($n = 10$) (WS: wheat straw; UTW: used tea wastes; OPC; olive pres cake; GP: grape pomace; GWH: green walnut hulls).

Yield and biological efficiency

Significant differences were observed among the tested media in terms of yield, and biological efficiency (BE) of *P. djamor* ($p < .001$) (Table 2).

The cultivation cycle of *P. djamor* lasted approximately 75 d, during which three to four flushes were harvested. The total fresh mushroom yield ranged from 152.6 g/kg to 213.0 g/kg, with the highest yield and BE (213 g/kg and 71.0%, respectively) observed in WS8:UTL2. Conversely, WS8:GWH2 recorded the lowest yield and BE (152.6 g/kg and 44.9%, respectively). While WS20:GWH2 had the lowest BE (44.9%), the media supplemented with UTL, OPC, and GP demonstrated satisfactory productivity, with BEs between 52.3% and 71.0%, similar to or higher than the control. For instance, UTL supplementation increased the total yield by 34% compared to the control, despite delaying the first harvest by 7 d. Similarly, media containing 20% GWH produced yields comparable to the control, though the time to fruiting was significantly extended.

Nutrient composition, total phenolic content and antioxidant activity

Table 4 reveals significant differences in parameters such as dry matter, ash, protein, crude fat, carbohydrate, and energy content of mushrooms obtained by cultivating *P. djamor* on different growing media ($p < .001$).

The dry matter content of the fruiting body varied significantly, ranging from 9.11 g/100 g to 11.42 g/100 g. In the present study, the protein content ranged from 15.17 g/100 g to 26.25 g/100 g, depending on the growing media. Notably, The protein content of mushrooms grown in the control medium was 23.3 g/100 g to 42.2 g/100 g higher than that of mushrooms cultivated on other media. However, increasing the N concentration did not always lead to higher protein levels in this study, with the exception of the GP treatment. Crude lipid content was relatively low (1.24–1.56 g/100 g), with the highest

Table 4. Effect of different growing media on the nutritional composition of *Pleurotus djamor* fruiting body.

Growing Media	Dry matter (g/100 g)	Ash (g/100 g)	Crude protein (g/100 g)	Crude fat (g/100 g)	Total carbonhydrate (g/100 g)	Energy (kcal/100 g)
Control	10.12 ± 0.21 d	7.63 ± 0.24 b	26.25 ± 0.06 a	1.24 ± 0.05 b	64.88 ± 0.24 e	359.50 ± 1.16 d
WS9:UTL1	10.77 ± 0.09 b	6.13 ± 0.08 d	20.13 ± 0.10 b	1.56 ± 0.05 a	72.17 ± 0.08 d	365.24 ± 0.32 a
WS8:UTL2	11.38 ± 0.14 a	6.14 ± 0.28 d	19.16 ± 0.09 cd	1.37 ± 0.12 ab	73.34 ± 0.32 cd	363.95 ± 0.46 abc
WS9:OPC1	9.59 ± 0.04 f	6.90 ± 0.12 c	18.43 ± 0.13 de	1.44 ± 0.09 ab	73.23 ± 0.10 bcd	361.33 ± 0.46 cd
WS8:OPC2	9.85 ± 0.06 e	6.96 ± 0.05 c	16.93 ± 0.07 f	1.46 ± 0.04 ab	74.65 ± 0.06 ab	360.81 ± 0.39 d
WS9:GP1	10.24 ± 0.09 d	8.77 ± 0.11 a	15.17 ± 0.02 g	1.25 ± 0.10 b	74.81 ± 0.22 a	352.45 ± 0.33 e
WS8:GP2	10.50 ± 0.12 c	6.39 ± 0.16 cd	19.45 ± 0.11 bc	1.25 ± 0.01 b	72.91 ± 0.07 cd	362.47 ± 0.64 bc
WS9:GWH1	9.21 ± 0.12 g	6.66 ± 0.20 cd	18.25 ± 0.36 e	1.42 ± 0.04 ab	73.68 ± 0.52 abc	362.03 ± 0.85 bc
WS8:GWH2	9.19 ± 0.12 g	6.42 ± 0.29 b	17.91 ± 0.46 e	1.43 ± 0.05 ab	74.24 ± 0.71 ab	362.93 ± 0.97 abc
p value		*<0.001*	*<0.001*	*0.002*	*<0.001*	*<0.001*

Mean values in the same column followed by the same letters are not significantly different by Tukey's test ($n = 3$) (WS: wheat straw; UTL: used tea leaves; OPC; olive pres cake; GP: grape pomace; GWH: green walnut hulls).

lipid content observed in mushrooms grown on WS9:UTL1, while the lowest was recorded in those cultivated on the control medium and GP-supplemented media. The ash content ranged from 6.13 g/100 g to 8.77 g/100 g, with the lowest values in UTL-supplemented media and the highest in WS9:GP1. Total carbohydrate content ranged from 64.88 g/100 g to 74.81 g/100 g, with WS:GP1 yielding the highest carbohydrate levels and the control medium the lowest. The energy values of mushrooms grown in different media varied between 352.45 kcal/100 g and 365.24 kcal/100 g dry weight., with *P. djamor* grown on UTL-enriched media showing higher energy values.

TPC, TEAC, and FRAP varied significantly with the growing medium ($p < .01$, Table 5). TPC values ranged from 21.72 to 34.41 mg GAE/g dry weight ($p < .001$), with the highest values observed in mushrooms grown in the control medium containing wheat bran and straw. In contrast, lower phenolic concentrations were found in mushrooms grown on media supplemented with phenolic-rich agro-industrial wastes such as OPC, GP, and GWH. TEAC values ranged from 12.53 µmol/g in WS9:OPC1 to 22.14 µmol/g in the control medium, which also exhibited the highest polyphenol content. FRAP values ranged from 16.35 µmol/g to 17.74 µmol/g, with the highest reducing potential in mushrooms from the control medium and the lowest in WS8:UT2. Similarly, the highest FRAP potential was found in mushrooms grown on control medium, while fruiting bodies grown on WS8:UT2 exhibited the lowest total antioxidant activities and low polyphenol content among the mushrooms tested. Significant correlations were found between TPC and TEAC ($r = 0.9699$) and TPC and FRAP ($r = 0.632$).

Fourier-transform infrared (FTIR) spectroscopic characterization

Fourier-transform infrared (FT-IR) spectroscopy was used to characterize the chemical composition of *P. djamor* grown on media supplemented with

Table 5. Effect of different growing media on the total phenolic content and antioxidant activity (ABTS and FRAP) of *Pleurotus djamor* fruiting body.

Growing Media	Total phenol (mg GAE/g dw)	TEAC (µmol TE/g dw)	FRAP (µmol TE/g dw)
Control	34.41 ± 0.57 a	22.14 ± 0.22 a	17.74 ± 0.10 a
WS9:UTW1	28.68 ± 0.95 b	17.12 ± 0.48 bc	17.11 ± 0.0 b
WS8:UTW2	25.91 ± 0.22 d	15.66 ± 0.30 cd	16.35 ± 0.17 d
WS9:OPC1	22.01 ± 0.37 e	12.53 ± 0.14 f	16.90 ± 0.25 bcd
WS8:OPC2	27.31 ± 0.22 bcd	16.10 ± 0.38 cd	17.09 ± 0.19 b
WS9:GP1	26.38 ± 0.62 cd	16.00 ± 0.90 cd	16.59 ± 0.16 bc
WS8:GP2	23.68 ± 0.12 e	15.30 ± 0.13 de	16.43 ± 0.04 cd
WS9:GWH1	21.74 ± 0.86 e	13.83 ± 0.19 ef	16.95 ± 0.24 bcd
WS8:GWH2	28.04 ± 0.74 bc	17.86 ± 0.60 b	16.68 ± 0.10 bcd
p value	<0.001	<0.001	<0.001

Mean values in the same column followed by the same letters are not significantly different by Tukey's test ($n = 3$) (WS: wheat straw; UTW: used tea wastes; OPC; olive pres cake; GP: grape pomace; GWH: green walnut hulls).

phenolic-rich agricultural wastes (Figure 1). The spectra ranged from 3282 cm^{-1} to 537 cm^{-1} and showed five regions: a fatty-acid region dominated by C – H bands (3450–2850 cm^{-1}), an amide region (1800–1500 cm^{-1}), a mixed region (1500–1200 cm^{-1}), a polysaccharide region (1200–900 cm^{-1}), and a fingerprint region (900–700 cm^{-1}). Overall spectral features were consistent across samples, with minor qualitative differences among mushrooms grown on different media.

A broad band at ~3300 cm^{-1} (within 3000–3500 cm^{-1}) was observed. Peaks at 2970 and 2930 cm^{-1} were present. High-intensity bands at approximately 1635, 1558, and 1374 cm^{-1} (amide I – III) were detected and were stronger in fruiting bodies from the control medium, particularly the amide II band. In the 1400–400 cm^{-1} region, peaks with maximum absorption at ~1030 cm^{-1} were recorded. The 900–1200 cm^{-1} interval showed prominent absorption,

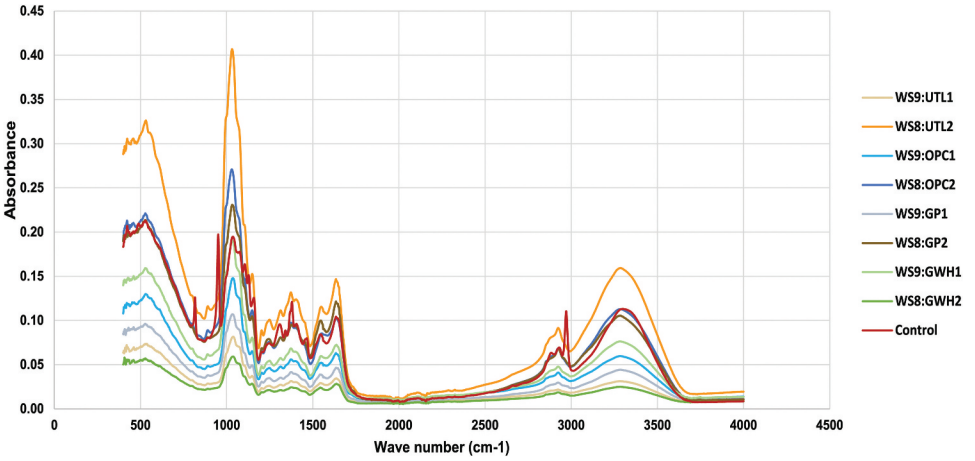

Figure 1. FTIR spectra of *Pleurotus djamor* cultivated on growing media supplemented with different ratios of phenolic-rich agro-industrial wastes.

and additional bands appeared around 1000–900 cm^{-1}. In the 950–750 cm^{-1} region, bands were observed at 890, 930, and 850 cm^{-1}; a band near 807 cm^{-1} was also present.

Discussion

Understanding the chemical composition of substrates is of great importance, as it directly determines their suitability for the cultivation of *Pleurotus* spp. Among the various physicochemical factors, moisture content plays a key role in fungal development, as it directly affects mycelial expansion and fruiting. While optimal moisture conditions promote growth, excessive or insufficient levels can adversely affect mushroom performance (Bellettini et al. 2019). In this study, the moisture content of the substrates ranged from 66.0% to 69.8%, which aligns with the optimal range of 60–70% reported in the literature (Abou Fayssal et al. 2020). The lower water retention in GP-, GWH-, and OPC-supplemented media can be attributed to the limited water-holding capacity of these materials compared to wheat bran.

The pH of the substrates is another important factor that affects fungal metabolism, as it regulates enzyme activity and nutrient bioavailability. In this study, the pH values ranged from 6.40 to 7.33, which is considered ideal for *Pleurotus* mycelial growth and fruiting body development (Drężek and Możejko-Ciesielska, 2025)). N supplementation has been reported in many studies to enhance the yield of *Pleurotus* spp. Although these fungi can grow on low-N substrates, N is a fundamental element required for protein, nucleotide, and chitin synthesis (Klimek and Piercey, 2024). However, excessive N levels may reduce yield and lower substrate utilization efficiency. Recent studies recommend maintaining substrate nitrogen availability within an optimal range (total $N \approx 1.8$–2.1% and $C{:}N \approx 26$–$30{:}1$) for *Pleurotus* spp. Levels outside this range, i.e., overly N-rich substrates or very low C:N tend to depress yield (Subedi et al. 2023; Wei et al. 2024). This indicates a favorable balance between N availability and substrate performance.

The C:N ratio is a critical determinant of mushroom productivity, as it governs N availability and carbon metabolism. Each *Pleurotus* spp. requires an optimal C:N ratio to achieve high yield and maintain an efficient cultivation cycle. Recent reviews indicate that an optimal C:N ratio for *Pleurotus* spp. lies around 45–60:1, with peak biological efficiency reported near 38–48:1; ratios outside this range tend to reduce performance (El-Ramady et al. 2022; Niloy et al. 2025; Silva et al. 2024; Zotti et al. 2025). In this study, the C:N ratio ranged from 46.1 to 95.6, which is consistent with values previously reported in the literature. However, the highest biological efficiency and yield were recorded in substrates with C:N ratios closer to the optimal range. This highlights the importance of maintaining a balanced C-to-N ratio for successful mushroom cultivation.

In this study, colonization occurred within 15.0–25.3 d, this interval is consistent with the 11.5–28 d reported in the literature, which vary with the biochemical properties of the lignocellulosic material, additive rates, and strain differences (İnci et al. 2024; Kılıç, 2020; Satpal, 2017). Similarly, the ranges obtained for pinhead formation and first harvest (19.1–33.6 and 23.6–39.6 d, respectively) overlap with the 16.67–35-d range reported in the literature (Hutabarat et al. 2022; Jegadeesh et al. 2018). The effect of OPC on SRP is evident. The addition of 20% OPC shortened colonization to as few as 15 d in some replicates and resulted in primordia appearing 7 d earlier than in the control (19.1 d). This finding is in agreement with reports that media containing 25% OPC mixed with sawdust support optimal mycelial development in *Pleurotus* spp (Atila, 2017). The C:N ratio – mycelial growth relationship observed in WS9:OPC1 and WS8:OPC2 is consistent with recent findings in *Pleurotus* that higher C:N ratios (within an appropriate range) accelerate colonization (Desisa et al. 2024; Silva et al. 2024). On the other hand, phenolic-rich residues appear to exert a dose-dependent delaying effect. Growing media containing 20% GWH delayed colonization by approximately 3–10 d relative to the control and WS8:OPC2; increasing UTL and GP to 20% extended colonization to 22.6 and 22.4 d, respectively. By contrast, in media containing 10% UTL, OPC, or GP (WS:UTL1, WS9:OPC1, WS9:GP1), the colonization time was observed to be even shorter than in the control. These delays observed at high inclusion levels of phenolic-rich wastes can plausibly be associated with phenolic constituents such as resveratrol in GP (Abedini et al. 2021), catechins/tannins in UTL (Ye et al. 2022), and juglone in GWH (Kithi et al. 2023). The longest pinhead and first-harvest times observed in WS8:GWH2 are likewise consistent with the allelopathic effect of juglone (Kithi et al. 2023).

Differences in yield across growing media are associated with the regularity of mycelial development and the extent to which the fungus can utilize nutrients in the substrate. A biological efficiency (BE) ≥50% is widely regarded as the minimum economically viable threshold for *Pleurotus* cultivation (Ahmed et al. 2024; Ritota and Manzi, 2019; Solórzano et al. 2025). While WS20:GWH2 had the lowest BE (44.9%), the media supplemented with UTL, OPC, and GP demonstrated satisfactory productivity, with BEs between 52.3% and 71.0%, similar to or higher than the control. In this study, the highest yield and biological efficiency were obtained in WS8:UTL2 (213.0 g/kg and 71.0% BE, respectively). This superiority can be attributed to the robust and homogeneous mycelial development observed under UTL supplementation and to a nutrient profile,consistent with a C:N ratio near the optimal range,that supports efficient substrate conversion. Notably, UTL increased total yield by 34% relative to the control despite a 7-d delay in the first harvest. Similarly, 20% GWH significantly delayed fructification while keeping yield close to the control. This pattern suggests that, although phenolic compounds (e.g.,

resveratrol in GP; catechins/tannins in UTL; juglone in GWH) slow early mycelial spread, total yield can increase if the formulation's overall nutrient content – particularly N and the C:N balance is adequate. Therefore, rather than categorically excluding phenolic-rich wastes, optimizing the dose and blend composition is a more rational approach.

The findings indicate the extent to which the substrate's chemical composition shapes the nutritional profile of the harvested fruiting bodies and underscore the importance of appropriate medium selection in mushroom cultivation. In the present study, the protein content ranged from 15.17 g/100 g to 26.25 g/100 g, depending on the growing media. Vega et al. (2022) reported similar protein levels (21.87–27.09 g/100 g) in *P. djamor*. Notably, *P. djamor* grown on wheat bran-based media showed significantly higher protein content than those grown on other growing media. This pattern is consistent with evidence that increased nitrogen availability via urea or nano-urea can modulate protein and amino-acid profiles as well as antioxidant capacity in *Pleurotus* spp (Begashaw and Tesfaw, 2025; Sassine et al. 2021). Nevertheless, in the present study, increases in N did not invariably translate into higher protein, with the GP treatment constituting an exception.

The genus *Pleurotus* is generally characterized by a high-protein/low-fat profile (Bellettini et al. 2019). Crude lipid content was low (1.24–1.56 g/100 g), the highest value was observed in WS9:UTL1, whereas the lowest values were recorded in the control and GP-supplemented media. The inverse relationship between protein and fat is consistent with the results of Bellettini et al. (2019). In addition, the lipid content of *P. djamor* has been reported to range from 0.1 g/100 g to 4.6 g/100 g in the literature, indicating a lower-fat profile than other mushroom species and, owing to its low fat and high unsaturated fatty-acid composition, a dietary advantage (Carrasco-González et al. 2017).

Ash content, reflecting mineral composition, ranged from 6.13 g/100 g to 8.77 g/100 g; the lowest values occurred in UTL-supplemented media and the highest in WS9:GP1. This distribution may be related to the mineral contents of the additive materials. Total carbohydrate content ranged from 64.88 g/100 g to 74.81 g/100 g. These levels are higher than the values reported by Carrasco-González et al. (2017) and Sultana et al. (2024). Energy content ranged from 352.45–365.24 kcal/100 g, enrichment with UTL was associated with relatively higher energy values. Taken together, these findings indicate that formulation composition meaningfully modulates macronutrient distribution and energy density.

The chemical composition of mushroom substrates also affects phenolic content and antioxidant activity (Atila et al. 2024; Diamantopoulou et al. 2023; Jimoh et al. 2023; Koutrotsios et al. 2017). In this study, TPC of *P. djamor* was consistent with the findings of Jayaseelan et al. (2024), who reported a value of 30.12 mg GAE/g. Interestingly, mushrooms grown on control media had higher TPC compared to those grown on agricultural wastes. This may be

due to wheat bran providing essential nutrients for polyphenol synthesis (Sardar et al. 2017), or because phenolic compounds in the substrates inhibit oxidase enzymes and chelate metal catalysts (Sufer et al. 2022).

In contrast, lower phenolic concentrations were found in mushrooms grown on media supplemented with phenolic-rich agro-industrial wastes such as OPC, GP, and GWH. An inverse relationship between productivity and phenolic content was also observed, as previously noted by Oropeza-Guerrero et al. (2018), who suggested that in highly productive environments, fungal metabolism is focused on growth rather than secondary metabolite production. Some previous studies also reported that no effects of phenolic rich by-products were found on polyphenol contents in *Pleurotus* spp., because the mushrooms could not absorb them (Diamantis et al. 2022; Melanouri et al. 2025). However, there are some studies in the literature reporting that the phenolic content of mushrooms grown on agricultural wastes with high phenol content may increase (Atila, 2017; Magdziak et al. 2021; Diamantopoulou et al. 2023; Atila et al. 2024). This may vary according to the mushroom species and may be due to the synergistic or antagonistic effects of N, lignocellulosic content and other compounds in the structure of the growing medium. More detailed studies are needed to reach a definite conclusion.

In the control medium, the highest TEAC and FRAP values coincided with the highest TPC, indicating that phenolics are the principal drivers of antioxidant capacity. The very strong TPC – TEAC correlation ($r = 0.9699$) and the moderate TPC – FRAP correlation ($r = 0.632$) indicate that phenolics are the primary contributors to antioxidant capacity. Accordingly, the very strong correlation between TPC and TEAC ($r = 0.9699$) and the moderate correlation between TPC and FRAP ($r = 0.632$) suggest a primary contribution of phenolic compounds, with non-phenolic reducing constituents (e.g., β-glucans) playing a secondary role on the FRAP side. The antioxidant levels obtained here exceed the value reported by Nattoh et al. (2016) for ripe *P. djamor* fruiting bodies (13.105 mg Trolox equivalent/g dry weight), indicating that formulation and nutrient inputs can elevate antioxidant output. A strong positive association between phenolic content and antioxidant activity in *Pleurotus* spp. has likewise been demonstrated by Peraza et al. (2019).

In the FT-IR spectra of the fruiting bodies, a set of diagnostic regions is apparent. The broad band at 3000–3500 cm^{-1}, centered near ~3300 cm^{-1}, reflects O – H stretching vibrations. This broad band reflects O – H stretching. The signal may originate from residual moisture or from the abundant hydroxyl groups of polysaccharides. O – H stretching of polysaccharide chains is typically observed in this region (Janjušević et al. 2017). Peaks at 2970 and 2930 cm^{-1} correspond to – CH stretching of lipids and carbohydrates (Koutrotsios et al. 2019). Prominent bands near ~1635 cm^{-1} (C = O stretching; amide I), ~1550 cm^{-1} (N – H bending; amide II) indicate proteins,

while the amide III band lies in the 1200–1400 cm^{-1} region and can appear around ~1370 cm^{-1} (C – N stretching/N – H bending) (Synytsya et al. 2023). Notably, the amide II band is stronger in the control samples, being consistent with higher protein levels and aligning with the measured crude protein contents across substrates. It should be noted, however, that intensities in this spectral window cannot be assigned to a single class of compounds: carbonyl vibrations in the 1670–1600 cm^{-1} range can arise from protein, phenolic, and flavonoid carbonyls (Piqueras et al. 2020). The higher absorption seen in the control samples may therefore also be compatible with greater antioxidant potential.

An absorption near ~1380 cm^{-1} is indicative of β-glucans (Ma et al. 2022). In the 1400–400 cm^{-1} region, peaks with a maximum around ~1030 cm^{-1} — characteristic of β-glucans – reflect C – O – C and C – O bonds within the D-glucose ring (Yan et al. 2018). Stronger absorption in the 900–1200 cm^{-1} polysaccharide region in the control samples points to a higher polysaccharide contribution, a feature consistent with bioactive properties attributed to mushroom polysaccharides and β-glucans (Yu et al. 2023). Bands around 1000–900 cm^{-1} correspond to C – C vibrations (alkanes) and C – H bending (alkenes), which are typical of major mushroom constituents (Rasika et al. 2022). In the 950–750 cm^{-1} interval, signals at ~890 cm^{-1} (C – H deformation of β-glucans) and ~930/~850 cm^{-1} (asymmetric ring vibrations and C – H deformation of α-glucans) map to the anomeric region of glucans. Bands near ~807 and ~930 cm^{-1} are associated with mannan – present in fungal cell walls – and chitin, the polymer of N-acetylglucosamine (Rasika et al. 2022).

The FT-IR data indicate relatively stronger protein and polysaccharide signatures in the control samples, the presence of anomeric markers for glucans, and overlap in carbonyl regions where protein and phenolic signals converge. Because FT-IR alone cannot fully resolve these components, complementary analyses – such as phenolic profiling by HPLC-DAD/LC-MS and quantitative assays of glucans – are recommended to refine molecular assignments.

Conclusion

The present results show that phenolic-rich agricultural by-products such as UTL, GWH, OPC, and GP can be effectively used in place of wheat bran for cultivating *P. djamor*. While wheat bran supported higher protein levels and stronger antioxidant activity, the addition of OPC accelerated mycelial growth and shortened the harvest period. UTL supplementation led to a clear increase in total yield, and FTIR analysis indicated that mushrooms grown on UTL-enriched media contained higher amounts of nutrients and bioactive compounds than those grown on the other media tested. Although some substrates caused slight reductions in certain nutrients, the overall contribution of

phenolic-rich residues was evident. These materials are low-cost, reduce waste, and support environmentally sustainable cultivation practices. Careful adjustment of substrate composition can improve not only the yield but also the functional compound profile of the mushrooms. In conclusion, UTL- and OPC-enriched substrates stand out as promising options for enhancing both productivity and quality in *P. djamor* cultivation, while contributing to more sustainable biotechnological applications. Future studies should evaluate the safety and nutritional value of the spent substrate derived from our formulations as a potential feed ingredient, thereby enabling double valorization. As an initial step, determining the carbohydrate/fiber fractions (e.g., NDF/ADF), mineral and heavy-metal contents, and the profiles of phenolic and antinutritional constituents associated with the residues used will directly inform the assessment of compliance with feed-safety thresholds.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

This manuscript includes all data relevant to the study.

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