Use of Oak Acorn Kernel (*Quercus ithaburensis* Decaisne)-Containing Substrates in Oyster Mushroom (*Pleurotus ostreatus*) Cultivation and Comparison with Traditional Growing Media

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This study aimed to evaluate the effects of different substrates containing oak acorn kernel on the cultivation of *Pleurotus ostreatus* for yield, biological efficiency (BE), and nutritional content. Different lignocellulosic wastes were used: Oak Acorn Kernel (AK), Oak Acorn Shell (AS), Oak Acorn Cup (AC), Hazelnut Branch (HB), Wheat Straw (WS), *Acacia* Branch (AB), Corncob (CC), and Oak Sawdust (OW). Substrates were prepared at 100% ratio and as 50% mixtures with WS; additionally, AK was supplemented with OW at 10%, 30%, and 50% ratios. Protein, phenolic content, and mineral compositions of mushrooms were analyzed. Chemical composition of substrates were revealed through chemical analyses. The highest mushroom yield was obtained from 50WS+50CC with 205.7 g/kg, while the maximum biological efficiency (57.6%) and benefit–cost ratio (4.98) were determined in the 50OW+50AK. The greatest phenolic content was found in mushrooms of HB substrate (58.781 mg GAE/g), the highest protein contents were detected in mushrooms grown on OW (28.43 g/100g) and AK (29.21 g/100g). Cu, Mn, P, and Zn elements were found to be higher in mushrooms of OW and AK substrates. This study makes a significant contribution to the circular economy by converting low-value forest wastes into value-added food products such as edible mushrooms.

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INTRODUCTION

The production of *Pleurotus ostreatus* (oyster mushroom) has rapidly increased in recent years. Commercial cultivation is particularly intensive in Southeast Asia, India, Europe, and Africa (Naim *et al*. 2020). Its economic return is considerably high due to the fact that it can be grown in all seasons of the year and on various lignocellulosic materials (Thangaraj *et al*. 2026). It ranks second among cultivated mushrooms worldwide, after *Agaricus bisporus* (Grimm and Wösten 2018). The main reasons for this mushroom's preference are its high protein content, low fat content, richness in vitamins, and its ability to grow on almost any type of lignocellulosic waste (Doroški *et al*. 2022). Thanks to its enzymes, such as laccase, cellulase, and peroxidase, it can easily break down biomass containing cellulose and lignin, such as wood, and convert it into a nutritious food product

(El-Ramady *et al*. 2022). According to studies, oyster mushroom (*Pleurotus ostreatus*) is rich in essential minerals such as potassium, phosphorus, calcium, iron, copper, zinc, magnesium, and selenium (Karataş 2022). Furthermore, oyster mushroom protein is also rich in essential amino acids of vital importance, such as leucine, lysine, and phenylalanine, which cannot be synthesized by the human body and must be obtained through diet (Effiong *et al*. 2024). Additionally, it has been reported that oyster mushroom possesses antiviral, antibacterial, antifungal, immunostimulatory, antioxidant, and antitumor effects (Elhusseiny *et al*. 2021).

Oyster mushroom can be successfully cultivated on various lignocellulosic substrates, such as wheat straw, rice straw, corncob, cotton waste, bamboo waste, sugarcane bagasse, or tree species including oak, poplar, and beech. According to Masevhe (2023), approximately 200 types of lignocellulosic waste can be used in edible mushroom cultivation. However, wheat straw, rice husk, and wood sawdust are the most commonly used substrates in mushroom cultivation (Miśkiewicz *et al*. 2025). However, the costs of these materials may increase due to economic challenges related to factors such as regional availability, seasonal variation, and logistical issues, *e.g.* transportation. Therefore, current research largely focuses on finding alternative, locally available, and low-cost waste materials to ensure sustainable mushroom production within the framework of the circular economy (Doroški *et al*. 2022). In contrast, the nutritional content of mushrooms also varies depending on the type of substrate on which they are cultivated (Singh *et al*. 2025).

Oak acorn kernels are currently mainly utilized as animal feed. However, a significant portion is left abandoned in fields without any processing. These underutilized products have become a new focus of interest due to concerns about food security and their potential health benefits (Martins *et al*. 2022). The inner part of the oak acorn (pelit or kernel) is exceptionally rich in starch (55%), fats, proteins, and essential minerals (Sassine *et al*. 2024).

Turkey has approximately 6.7 million hectares of oak forests (OGM, 2022). These forests include multiple oak species that produce acorns with similar nutritional profiles (starch, protein, and minerals), suggesting that the potential resource base for kernel-based applications extends well beyond a single species. The annual acorn production potential is estimated to be equivalent to 20 million tons of wheat or 6.7 million tons of barley, yet current harvest remains around 15,000 tons per year. In 2023, under the Oak Acorn Action Plan, 2,220 tons of acorns were produced. Valex (acorn extract) production ranges between 1,300 to 1,500 tons annually, contributing approximately 3 million USD to the national economy. Beyond this established use, the acorn kernel itself-currently underutilized as low-value feed or left as waste-offers an innovative opportunity for valorization in edible mushroom cultivation within a circular economy framework.

In many countries, oak sawdust is used as the main substrate in mushroom cultivation, which puts pressure on oak forests. For this reason, mushroom growers use substrates that are easily available in their regions and prefer lignocellulosic materials that meet the needs of the mushroom species they cultivate. The direct use of nutritionally rich oak acorn kernel as a substrate component for *P. ostreatus* fruiting bodies has not yet been investigated. Although the kernel is not yet collected on an industrial scale for mushroom cultivation, Turkey's extensive oak forests and the existing acorn collection infrastructure suggest that this resource could be mobilized if economically viable applications are demonstrated. The national 'Oak Acorn Action Plan' (OGM, 2022) actively supports such diversification. While Sassine *et al*. (2024) conducted a study on shiitake mushroom

cultivation using oak acorns, there is no study in the literature on the yield and quality of oyster mushrooms in this regard.

In this study, oak acorn kernels were, for the first time, evaluated as substrates together with oak sawdust for *P. ostreatus* cultivation. They were compared with conventional lignocellulosic materials used in traditional production methods, such as wheat straw, corncob, oak acorn cup, acacia branch, and hazelnut branch. Additionally, the nutritional contents of the mushrooms were analyzed, and their mineral content, antibacterial properties, protein, and total phenolic contents were determined. In contrast, the holocellulose, alpha-cellulose, and lignin contents of the substrates used were determined to assess their suitability for mushroom cultivation and their structural characteristics. Furthermore, economic return analyses were conducted to reveal the commercial profitability status.

EXPERIMENTAL

Preparation of Substrates

In the study, the following materials were used as substrates: Oak acorn Kernel (AK), oak acorn sell (AS), oak acorn cup (AC), hazelnut branch (*Corylus avellana* L.) (HB), wheat straw (WS), acacia branch (*Acacia* sp.) (AB), corncob (CC), and oak sawdust (*Quercus* sp.) (OW). Hazelnut branches, corncobs, and wheat straw were obtained from local producers in the Düzce region. Acacia branches were obtained from pruning waste within the Düzce University campus. Oak acorns were supplied from the Ar-Tu Kimya San. ve Tic. A.Ş. factory located in Salihli/Manisa, Turkey. The wastes (AK, AS, AC, HB, AB, CC, and OW) were ground in a Wiley mill to approximately 0.5 to 1 cm, while WS was ground to approximately 5 cm in size. For the removal of tannins and other phenolic compounds, oak acorn kernels (AK) were pretreated as follows: 500 g of ground AK was soaked in 3 L of hot water (70 to 80 °C) for 1 h, followed by filtration through a strainer. The residue was then soaked in 3 L of tap water at room temperature (24 °C) overnight (approximately 16 h), filtered again, and air-dried at 40 ± 2 °C for 48 h until constant weight. The pretreated AK was then used for substrate preparation. It should be noted that residual tannin/phenolic content was not measured after pretreatment; therefore, the complete removal of tannins was not verified. This is acknowledged as a limitation of the study.

From the substrates, homogeneous air-dry mixtures were prepared by weight (w/w) at 100% ratio and as 50% mixtures with wheat straw (Table 1). Additionally, AK was supplemented to OW material at 10%, 30%, and 50% ratios to create mixtures. Thus, the effect of AK on mushroom cultivation in OW material was determined. After adding 1% lime (calcium carbonate) to the mixtures based on dry weight, they were moistened at regular intervals over the course of one day. Then, 1 kg from each combination was weighed, filled into heat-resistant polypropylene bags (28 × 42 cm), and sterilized in an autoclave at 121 °C for 1.5 h. At least 6 replicates were autoclaved for each combination. After sterilization, the pH and moisture contents of each mixture were determined. The autoclaved composts were left to cool overnight and allowed to reach room temperature (24 °C). Subsequently, in a sterile air cabinet, *P. ostreatus* mycelium was inoculated into the substrates at a rate of 3% of the wet compost weight (30 g spawn per 1 kg wet substrate; 15 g per 500 g wet AK and its mixture substrates). The same inoculum rate was applied to all treatments. The *P. ostreatus* mycelium used in the study was obtained from Erkel

Company (İzmir, Turkey). The mycelium-inoculated substrates were mixed homogeneously, the mouths of the bags were tightly plugged with cotton, and they were transferred to the climate-controlled room (incubation room).

Two different bag sizes and moisture contents were used, depending on the substrate type. For conventional substrates and their mixtures (100WS, 100HB, 100CC, 50WS+50HB, *etc*.), 1 kg of substrate at 40% moisture content was filled into each bag, resulting in 600 g dry matter per bag. For oak acorn kernel (AK) and its mixtures with OW, 0.5 kg of substrate at 50% moisture content was used to ensure uniform mycelial colonization due to the dense and fine texture of AK, yielding 250 g dry matter per bag. To standardize yield comparisons, fresh yield from 0.5 kg bags was multiplied by 2 and expressed as g/kg wet substrate. However, biological efficiency (BE) was calculated on a per-bag basis using the actual dry weight, without any multiplication, because BE is a ratio independent of bag size.

$$\text{Yield} = \text{Fresh mushroom weight (g)} / \text{weight of wet substrate (1kg)} \quad (1)$$

$$\text{BE} = \text{Fresh mushroom weight} / \text{initial dry substrate weight} \times 100. \quad (2)$$

Table 1. Mushroom Cultivation Substrates

Substrates	Symbol	Replicates	pH
Wheat Straw 100%	100WS	7	7.36
Hazelnut Branch 100%	100HB	12	7.15
Corncob 100%	100CC	6	6.15
Oak Acorn Cub 100%	100AC	6	6.42
Oak Acorn Shell 100%	100AS	6	6.40
Acacia Branch 100%	100AB	6	6.72
Wheat Straw 50% + Corncob 50%	50WS+50CC	6	6.42
Wheat Straw 50% + Oak Acorn Shell 50%	50WS+50AS	6	6.82
Wheat Straw 50% + Oak Acorn Cub 50%	50WS+50AC	6	6.90
Wheat Straw 50% + Hazelnut Branch 50%	50WS+50HB	6	6.37
Wheat Straw 50% + Acacia Branch 50%	50WS+50AB	6	7.05
Oak Acorn Kernel 100%	100AK	6	4.90
Oak Sawdust 100%	100OW	6	5.33
90% Oak Sawdust + Oak Acorn Kernel 10%	90OW+10AK	6	5.26
Oak Sawdust 70%+ Oak Acorn Kernel 30%	70OW+30AK	6	5.16
Oak Sawdust 50%+ Oak Acorn Kernel 50%	50OW+50AK	6	5.04

pH values measured after lime supplementation (1% $CaCO_3$) and moisture conditioning

For each treatment, at least 6 replicate bags (polypropylene, 28×42 cm) were prepared initially. These bags were considered as independent experimental units. In the incubation room (24±2°C, 70% relative humidity), the bags were randomly arranged; however, their positions were not swapped to minimize potential spatial gradients in temperature, light, and air circulation. All treatments were conducted simultaneously in a single climate-controlled room; therefore, no independent experimental run was performed. This constitutes a major limitation of the study, and the results should be interpreted as specific to the conditions of this single trial.

The species identification was based on the supplier's declaration and confirmed by morphological examination of the acorns (oval shape, 4 to 5 cm in length, with a cup covering approximately one-third of the acorn and densely covered with thick, recurved scales). The acorns were harvested in autumn 2024 from naturally growing trees in the Salihli region (Manisa, Türkiye). No voucher specimen was deposited. Oak sawdust

(*Quercus* sp.) was obtained from a local sawmill in Düzce, Türkiye, and was used as a conventional control substrate due to its known suitability for *P. ostreatus* cultivation. It should be noted that the oak sawdust and oak acorns were obtained from different sources and do not originate from the same tree species. The commercial strain of *Pleurotus ostreatus* (Jacq.) P. Kumm was obtained from Erkel Company (İzmir, Türkiye). The strain was supplied as grain spawn on a rye grain medium. Upon arrival, the spawn was stored at 4 °C and activated by incubation at 24 °C for 48 h prior to inoculation.

Incubation and Harvest

The substrates were kept in a climate-controlled room at 24 ± 2 °C temperature and 70% relative humidity in a dark environment. After mycelial colonization was completed, holes were opened on the bags, and the room temperature was adjusted to 16 ± 2 °C. The room humidity was increased to 80 to 90%. Following this stage, the incubation room was ventilated using a timer-controlled exhaust fan, and CO_2 concentration was monitored with a CO_2 sensor to keep levels below 1000 ppm. Humidity was measured and controlled using a digital hygrometer and a humidifier system. After primordium formation was observed, cool white fluorescent light (50 to 60 lux) was provided to the room to stimulate cap development. The lights were operated on a 9/15 h light/dark photoperiod. The room was ventilated automatically with a mechanical ventilation system set to operate for 30 minutes every 1.5 hours (*i.e.*, 16 air exchanges per day). The entire fruiting body (including both cap and stem) were collected and weighed on a precision balance and their fresh weights were recorded. Depending on the mixture type, a total of 2 or 3 flushes (harvest periods) were collected from each combination. For each treatment, harvesting continued until no new primordia appeared after three consecutive flushes, with a maximum of three flushes harvested per bag. Contaminated bags were excluded from the analysis. Total yield was calculated as the sum of all flushes per treatment For each combination, the yield, biological efficiency (BE), dry weight of mushroom samples, mycelial colonization time, primordium formation time, first harvest time, and total harvest time were calculated.

Nutritional Content Analysis of Mushrooms

For nutritional analyses, composite samples were prepared by pooling fruiting bodies from at least three culture bags per treatment. All analyses were performed on these composite samples. Total phenolic content analyses were carried out in triplicate, while protein determination was performed in single replicate.

Determination of total phenolic content

One gram of dried and ground mushroom sample was weighed and subjected to ultrasonic bath-assisted extraction with 10 mL of ethanol for 1 h. The extract was filtered through a 0.45-µm membrane filter, and the filtrate was collected for total phenolic content analysis. The total phenolic content was determined using the Folin–Ciocalteu spectrophotometric method. Absorbance measurements were performed at 760 nm using a Shimadzu UV-1800 spectrophotometer. A stock solution of gallic acid, used as the standard, was prepared in methanol at a concentration of 1000 ppm, and then standard working solutions were obtained through serial dilutions. The reaction mixture consisted of 200 µL of sample (or standard or blank), 1000 µL of 0.5 N Folin–Ciocalteu reagent, and 800 µL of 10% Na_2CO_3 solution, brought to a final volume of 3000 µL with distilled water. The tubes were vortexed and incubated at room temperature for 30 min, after which their absorbances were recorded. The blank was prepared using distilled water instead of the

extract. Gallic acid calibration standards were prepared at concentrations of 15.625, 31.25, 62.5, 125, 250, and 500 mg/L, yielding the regression equation y = 0.00154x – 0.00621 (R^2 = 0.99875). Samples were diluted 1:10 when necessary. Results were calculated as mg gallic acid equivalents (GAE) per gram of dried mushroom powder (mg GAE/g) (Singleton *et al*. 1999).

Nitrogen and protein determination

The Kjeldahl method was used to determine the total nitrogen and crude protein content in the mushroom caps. Analytically weighed mushroom powder samples were subjected to wet digestion in Kjeldahl tubes with concentrated sulfuric acid and catalyst tablets in a digestion unit at 420 °C until a clear solution was obtained, thereby ensuring that all nitrogen in the organic structure was converted to ammonium sulfate. After the digestion process, the cooled solutions were alkalinized with 40% sodium hydroxide in an automatic distillation unit, and the released ammonia gas was collected in a boric acid solution with the aid of water vapor. The amount of ammonia accumulated in the collection solution was titrated with a standard 0.1 N hydrochloric acid solution, and the total nitrogen content (%) of the samples was calculated. The crude protein contents on a dry weight basis were then determined by multiplying the obtained nitrogen values by the protein conversion factor of 4.38 Nitrogen and protein determinations were carried out in single replicate. Results are presented accordingly in Table 5.

Elemental analysis of mushrooms

Elemental analysis of the dried and ground mushroom fruitbody was performed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; Avio 200; PerkinElmer, Waltham, USA). Dried mushroom samples (0.25 g) were digested in a microwave system (CEM MARS6) with 10 mL of 67% HNO_3 (180 °C, 20 min), diluted to 25 mL with ultrapure water, and analyzed by ICP-OES (SPECTRO BLUE II) under the following conditions: 1200 W, coolant 13 L/min, auxiliary 0.8 L/min, nebulizer 0.8 L/min, and cyclonic spray chamber. Multi-element standards (0.1–10 mg/L) were used for calibration, and accuracy was verified with NIST SRM 1570a (recoveries 92–105%). Reagent blanks were used for correction; LOD and LOQ were calculated as 3σ and 10σ of blank signals. All measurements were performed in triplicate

Chemical Analysis of Substrates

The chemical analyses of the substrates used in the study were performed as follows: holocellulose content was determined according to Wise and Jahn (1952), alpha-cellulose content according to TAPPI T203 cm-09 (2009), and ash content and organic carbon according to TAPPI T211 om-16 (2022).

To isolate holocellulose, 5 g of 40-mesh samples-previously oven-dried at 103 ± 2°C-were processed inside 250 mL Erlenmeyer flasks using a four-step delignification procedure. Each individual 1-hour cycle involved mixing the sample with 160 mL of distilled water, 1.5 g of sodium chlorite ($NaClO_2$), and 0.5 mL of glacial acetic acid. This mixture was maintained at 78 ± 1°C in a stirred water bath and shaken vigorously every 15 minutes to maintain homogeneity. At the end of each hour, the reaction was replenished with fresh $NaClO_2$ (1.5 g) and acetic acid (0.5 mL), repeating the sequence four times for complete lignin extraction. The isolated holocellulose was then recovered *via* vacuum filtration using tared porosity-2 glass crucibles, washed thoroughly with chilled distilled water and acetone, and dried at 103 ± 2°C until a stable weight was reached. Finally, the

total holocellulose yield (%) was calculated based on the initial oven-dry mass of the substrate.

Lignin content was not determined directly but was calculated as the difference between 100% and holocellulose content (Lignin percent = 100 – Holocellulose percent), as is commonly practiced in preliminary substrate characterization studies (Wise and Jahn, 1952).

Based on a modified TAPPI T 203 cm-09 protocol, α-cellulose quantification was performed in triplicate using 2 g of holocellulose. The substrate was initially exposed to 17.5% NaOH (10 mL initially, then two 5 mL increments every 5 min) for 30 min at 20.0 ± 0.5°C. The mixture was diluted with 33 mL of distilled water, left to stand for 60 min, and then filtered *via* porosity-2 crucibles. Purification of the residue involved sequential washing steps with 8.3% NaOH (100 mL), 10% acetic acid (15 mL), and distilled water (250 mL). After drying the clean residue to a constant weight at 105°C, the α-cellulose fraction was determined gravimetrically relative to the initial sample mass.

Statistical Analyses

One-way analysis of variance (One-Way ANOVA) was used to reveal the differences among the mean yield, biological efficiency, dry weight and percentages of mushrooms obtained from the substrates used in the study, as well as the elemental analysis and chemical analysis values of the substrates (holocellulose, alpha-cellulose, lignin, ash, and organic carbon). Duncan's test was applied to separate the means at a significance level of $\alpha = 0.05$ using IBM SPSS Statistics (IBM Corp., Version 22.0, Armonk, NY, USA).

Economic Return Analysis of Mushrooms According to Growing Medium

The following formulations were used in the economic return analysis of the mushrooms cultivated in this study (Thangaraj *et al*. 2026). The selling price of mushrooms was taken as 450 ৳ per kg:

$$\textit{Gross Income} = \textit{Total fresh mushroom yield} \times \textit{Mushroom selling price}/1000 \quad (3)$$

$$\textit{Net Income} = \textit{Gross Income} - \textit{Total production cost} \quad (4)$$

$$\textit{Benefit–Cost Ratio (B:C)} = \textit{Gross Income} / \textit{Total production cost} \quad (5)$$

RESULTS AND DISCUSSION

The holocellulose, alpha-cellulose, lignin, ash, and organic matter contents of the substrates are presented in Table 2. Among the substrates, the highest holocellulose content (80.1%) and the lowest lignin ratio (19.9%) were found in the 100OW substrate. This value was statistically significantly different from the other substrates ($p < 0.05$). This result reveals that the 100OW substrate was found to be the most suitable substrate for mushroom cultivation. The lowest holocellulose ratio (54.3%) and the highest lignin ratio (45.7%) were determined in the AS substrate. Similar findings were observed for 100AC among the oak acorn fractions. Regarding alpha-cellulose content, the highest value was determined in AS with 69.3%, while the lowest was found in HB with 47.3%. These findings indicate that 100AS and 100AC negatively affected mycelial development and reduce mushroom yield due to their high lignin and alpha-cellulose (crystalline cellulose) content. Alpha-cellulose, due to its crystalline structure and degree of polymerization, is

less accessible for degradation by mushroom enzymes (Adeleye *et al*. 2022). In this study, as alpha-cellulose contents increased, the yield values of mushroom composts decreased. For example, substrates with high alpha-cellulose content (100AS and 100AC) exhibited significantly lower yields (31.2 to 32.5 g/kg) and biological efficiency (5.2 to 5.4%). In contrast, substrates with low alpha-cellulose content, such as 100HB (47.3%), 100WS (54.9%), and 100OW (55.4%), yielded higher values (173.8, 162.8, and 120.6 g/kg, respectively). A Pearson correlation analysis was performed to examine the relationship between α-cellulose content and mushroom yield using the mean values for each substrate type (n = 6). The analysis revealed a strong negative correlation (r = –0.752), indicating that substrates with higher α-cellulose content tended to have lower yields. However, this correlation was not statistically significant (p = 0.084), likely due to the limited number of substrate types (n = 6). Therefore, while the observed trend supports a potential negative association between α-cellulose and yield, this relationship should be interpreted with caution and requires further validation with a larger sample size. When ash and organic matter contents were examined, statistically significant differences emerged among all substrates ($p < 0.05$). The highest inorganic matter was determined in the 100WS substrate with 7.24%. The high inorganic matter content in the 100WS substrate can be attributed to the amorphous silica (SiO_2) present in its structure (Ramasamy *et al*. 2023). For oak acorn kernel (100AK), the methods used are not suitable for elucidating cellulosic content due to its high starch content rather than cellulose and lignin. However, the literature indicates that oak acorn kernel (100AK) contains 55% starch (Sassine *et al*. 2024). Therefore, this high starch content demonstrates that it is a suitable substrate for mushroom cultivation.

Table 2. Chemical Composition of Substrates

Substrates	Holocellulose (%)	Lignin (%)	α Cellulose (% of holocellulose (%)	Ash (%)	Organic Matter Content (%)
100AS	54.3 (0.37) *a*	45.7 (0.37) *a*	69.3 (1.44) *a*	2.44 (0.69) *a*	97.6 (0.06) *a*
100AC	67.8 (0,22) *b*	32.2 (0.22) *b*	59.6 (2.21) *b*	3.04 (0.27) *b*	97.0 (0.27) *b*
100AB	69.0 (1.04) *c*	31.0 (1.04) *c*	52.6 (0.77) *c*	4.04 (0.14) *c*	96.0 (0.14) *c*
100AK	nd	nd	nd	3.93 (0.32) *c*	96.1 (0.32) *c*
100HB	74.0 (0.13) *d*	26.0 (0.13) *d*	47.3 (0.28) *d*	2.49 (0.32) *a*	97.5 (0.32) *a*
100OW	80.1 (0.26) *e*	19.9 (0.26) *e*	55.4 (0.40) *e*	1.89 (0.16) *d*	98.1 (0.16) *d*
100WS	74.6 (0.71) *d*	25.4 (0.71) *d*	54.9 (0.22) *e*	7.24 (0.19) *e*	92.8 (0.20) *e*

Holocellulose, lignin, and α-cellulose values are expressed as percentages of the original dry matter, except for α-cellulose, which is expressed as a percentage of the holocellulose fraction. Lignin values were calculated as the difference between 100% and holocellulose content (Lignin = 100 − Holocellulose). α-Cellulose was determined on the holocellulose residue according to TAPPI T203 cm-09. Different letters in the same column indicate statistically significant differences between the means ($p < 0.05$). Values in parentheses are standard deviation; nd: not detected by this method

Although pH values differed among substrates (Table 1), all values (4.90 to 7.36) were within the generally accepted range for *P. ostreatus* cultivation (Bellettini *et al.* 2019). The standardized addition of 1% lime to all substrates ensured baseline pH adjustment while allowing the natural characteristics of each material to be evaluated. The pH values of the substrates ranged between 4.90 and 7.36 after lime supplementation. While *P. ostreatus* generally tolerates a wide pH range (5.0 to 7.5), optimal mycelial growth and fruiting body formation typically occur near neutral pH (Bellettini *et al.* 2019). The lower pH values observed in oak-based substrates (4.90 to 5.33) did not appear to adversely affect mushroom yield or biological efficiency, as evidenced by the high performance of OW and

AK-containing mixtures. This suggests that the inherent acidity of oak materials is well-tolerated by *P. ostreatus*, possibly due to the mushroom's adaptation to woody substrates. However, the extremely low yield and biological efficiency observed in 100AC (pH 6.42) and 100AS (pH 6.40) cannot be attributed solely to pH, as these values fall within the acceptable range for *P. ostreatus* cultivation. Rather, the cellulose and lignin contents of these materials are also considered to be the primary limiting factors.

The mean yield, mean biological efficiency, and dry weight values according to substrate type are presented in Table 3. After mushroom harvest, the differences among the mean yield values of the substrates used in the study were found to be statistically significant ($p < 0.05$). Among the mixtures prepared with 100% substrates, the highest yield value was observed in 100CC with 197.1 g/kg, while the lowest yield value was observed in 100AS with 31.2 g/kg. In the mixtures supplemented with 50% wheat straw, the highest yield value was obtained in 50WS+50CC with 205.7 g/kg, whereas the lowest yield value was determined in the 50WS+50AB mixture with 81.3 g/kg, and the difference among the means was statistically significant ($p < 0.05$). In mushrooms cultivated with oak sawdust supplemented with oak acorn kernel at 10%, 30%, 50%, and 100% ratios, the yield was lowest in 100% oak acorn kernel (68.7 g/kg) and highest in the 50OW+50AK mixture with 143.9 g/kg. No statistically significant differences were observed among the yield values obtained from both the oak control group (100OW) and the oak acorn kernel additions ($p > 0.05$).

When the biological efficiency (BE) data were examined, generally higher results were obtained in mixtures containing oak and oak sawdust supplemented with acorn kernel. The highest BE value of 57.6% was obtained in the 50OW+50AK mixture. While this value was not statistically significantly different from 100OW and the other AK-supplemented combinations ($p > 0.05$), it was significantly different from the other 100% substrates (100WS, 100HB, 100CC, 100AC, 100AS, 100AB) and from all wheat straw mixtures ($p < 0.05$). Among the BE values, the lowest values were observed in 100AS and 100AC substrates with 5.4% and 5.2%, respectively. Statistically significant differences were found among the mean dry weight percentages of mushrooms obtained from the substrates ($p < 0.05$). The highest dry weight percentage was determined in the 100AC substrate (32.0%), which was significantly higher than the other substrates. Among the 100% substrates, 100AC showed the highest dry matter content percentage. When oak sawdust and acorn combinations were evaluated separately, the dry weight of mushrooms obtained from 100AK (24.0%) was higher than the other groups and the difference was statistically significant.

When the yield values were compared with the literature, high yield values were obtained in traditional cultivation substrates (WS, CC, OW, HB) as expected. Fufa *et al*. (2021) reported the yield of *P. ostreatus* on corncob substrate as 144.18 g/kg, which is close to the yield value of 197.1 g/kg obtained in the current study. Similarly, Akcay *et al*. (2023) reported yield values of 255.7 g/kg on hazelnut branches (100HB) and 125 g/kg on a wheat straw and hazelnut branch mixture, while the findings in this study were determined as 173.8 g/kg and 125.8 g/kg, respectively. In traditional production substrates, biological efficiency values in this study were lower compared to the literature. However, in the acorn-supplemented oak sawdust, biological efficiency values were found to be quite close to the literature data. In a study conducted by Shah *et al*. (2004), the highest biological efficiency (BE) value for *Pleurotus ostreatus* was reported as 64.69% on oak sawdust. In the same study, the BE value of wheat straw was found to be 44.72%, and the BE value of the 50% sawdust + 50% straw mixture was 43.59%. In this study, the BE value of oak

sawdust was 48.3%, which is lower than the BE value found by Shah *et al*. (2004), while the BE values in 90OW+10AK and 50OW+50AK oak acorn kernel (AK) supplementation were determined as 57.4% and 57.6%, respectively, and these values are quite close to those reported in the literature.

Oak acorn kernel contains 55% starch (Sassine *et al.* 2024), which may serve as a readily available carbon source when hydrolyzed by fungal amylases. This mechanism may be relevant at lower AK concentrations; however, at higher concentrations (*e.g*., 50%), physical factors such as substrate compaction and reduced aeration may override this potential benefit and delay mycelial spread.

Table 3. Yield, Biological Efficiency, and Dry Weight Values According to Substrate Type

Substrates	Mean Yield (g/kg)	Biological Efficiency (BE) (%)	Mean Dry Mushroom Weight (g)	Mean Dry Mushroom Weight Percentage (%)
100WS	162.8 (18.33*) *bcd*	27.1 (8.1**) *abc*	21.8 (4.5**) *def*	14.0 (3.1) *ab*
HB100	173.8 (11.59) *cd*	29.0 (10.0) *bc*	23.5 (8.4) *ef*	13.6 (2.4) *ab*
100CC	197.1 (9.41) *d*	32.9 (2.7) *bc*	37.1 (9.0) *g*	18.7 (3.5) *bc*
100AC	32.5 (14.56) *a*	5.4 (3.4) *a*	10.0 (5.4) *abc*	32.0 (3.5) *d*
100AS	31.2 (6.44) *a*	5.2 (2.1) *a*	3.9 (1.8) *a*	12.9 (3.8) *ab*
100AB	132.2 (7.67) *bcd*	22.0 (1.8) *ab*	18.1 (4.4) *bcde*	13.9 (4.5) *ab*
50WS+50CC	205.7 (33.35) *d*	34.3 (11.1) *bc*	31.7 (9.1) *fg*	15.9 (3.9) *ab*
50WS+50AS	120 (30.31) *abcd*	20.0 (14.3) *ab*	12.8 (7.7) *abcde*	11.4 (3.3) *a*
50WS+50AC	123.7 (10.20) *abcd*	20.6 (2.9) *ab*	20.3 (8.3) *cde*	17.0 (9.0) *ab*
50WS+50HB	125.8 (4.81) *abcd*	21.0 (1.1) *ab*	16.8 (1.4) *bcde*	13.4 (0.4) *ab*
50WS+50AB	81.3 (5.47) *abc*	13.6 (1.3) *ab*	11.7 (2.4) *abcd*	14.7 (4.5) *ab*
100AK	68.7 (11.93) *ab*	27.5 (11.7) *abc*	7.4 (1.6) *ab*	24.0 (9.0) *c*
100OW	120.6 (6.44) *abcd*	48.3 (6.3) *cd*	8.2 (0.7) *ab*	13.8 (2.0) *ab*
90OW+10AK	143.5 (26.74) *bcd*	57.4 (26.2) *d*	10.5 (3.4) *abc*	15.5 (2.8) *ab*
70OW+30AK	119.8 (11.52) *abcd*	47.9 (9.2) *cd*	9.3 (0.7) *abc*	15.9 (2.5) *ab*
50OW+50AK	143.9 (32.71) *bcd*	57.6 (29.3) *d*	11.1 (4.5) *abcd*	16.3 (3.2) *ab*

*Values in parentheses are standard errors. ** Values in parentheses are standard deviations. Statistical analysis (Duncan's test) was performed separately for each substrate block: 100% substrates, 50% wheat straw mixtures, and AK-supplemented groups. Different letters within the same column indicate statistically significant differences ($p < 0.05$). Substrate abbreviations: WS: wheat straw; HB: hazelnut branch; CC: corncob; AC: oak acorn cup; AS: oak acorn shell; AB: acacia branch; OW: oak wood sawdust; AK: oak acorn kernel

Table 4 presents the effects of oak acorn kernel (AK) supplementation on mycelial colonization time, primordium formation time, first harvest time, and total harvest time. When the colonization times were examined, the lowest mean colonization time was observed in 100AK with 13 days, while the highest mean colonization time was observed in the 50OW+50AK substrate with 16.8 days. The differences among the means were found to be statistically significant ($p < 0.05$), with a 29.2% delay in colonization time. Similar differences were observed in primordium formation time. For the first harvest time, the shortest harvest time was found in the 100AK substrate, and no statistically significant differences were found among the other combinations except for 50OW+50AK ($p > 0.05$). Regarding total harvest times, the shortest harvest time was found in 100OW with 35 days,

while the longest was determined in 50OW+50AK with 54.8 days. Although delays were observed in the 50OW+50AK combination, no statistically significant differences were found in total harvest times ($p > 0.05$). While AK supplementation did not negatively affect total yield, the early growth delay observed at high AK concentrations (50%) suggests that the dense and compact structure of AK may limit aeration and slow mycelial penetration during the early colonization phase. The dense and fine texture of AK, combined with its high starch content, may create a compact substrate matrix that restricts aeration and slows hyphal penetration during the initial colonization phase. Similar findings have been reported for other dense agricultural wastes used in mushroom cultivation (Doroški *et al.* 2022). Therefore, while AK is a promising substrate component, its proportion should be carefully optimized to balance early colonization and overall yield performance. Future studies should investigate the physical properties of AK-based substrates, such as bulk density and porosity, to better understand their effects on mycelial growth. In substrates prepared as 100% AC and 100% AS, mushroom yields remained quite low. However, when they were added to wheat straw at 50%, yield values increased. This shows that they can be used not as 100% but rather as supplements to wheat straw.

The effect of AK supplementation on mycelial colonization time, primordium formation time, first harvest time, and total harvest time has also been demonstrated in previous studies. Sassine *et al*. (2024), in their study on shiitake mushroom (*Lentinus edodes*), reported that compared to oak sawdust, primordium formation time decreased from 75 days to 65 days and first harvest time decreased from 79 days to 70 days.

Table 4. Effect of Oak Acorn Kernel Supplementation on Colonization and Harvest Time

Substrate Types	Spawn Run Time (Days)	Primordium (Days)	First Harvest Day (Days)	Total Harvest Day (Days)
100AK	13 (0.00) *a*	23.3 (4.17) *a*	29.2 (2.85) *a*	48.3 (16.6) *a*
100OW	14.3 (0.8) *a*	24.3 (2.16) *a*	30.0 (2.00) *a*	35.3 (12.7) *a*
90OW+10AK	14 (1.41) *a*	25.0 (3.00) *a*	31.6 (3.73) *ab*	45.3 (17.2) *a*
70OW+30AK	13 (0.00) *a*	24.3 (4.04) *a*	32.0 (3.46) *ab*	50.0 (18.1) *a*
50OW+50AK	16.8 (1.6) *b*	30.0 (4.47) *b*	35.8 (4.02) *b*	54.8 (19.0) *a*
The same letters in the same column indicate that there is no statistically significant difference between the means. Values in parentheses are standard deviation.				

To evaluate the dose-response effect of oak acorn kernel (AK) addition, separate one-way ANOVAs were performed for the AK-containing treatment groups (100OW, 90OW+10AK, 70OW+30AK, and 50OW+50AK) across multiple parameters, including yield, biological efficiency (BE), spawn run time, primordium formation time, first harvest time, and total harvest time. Levene's test indicated a significant violation of the homogeneity of variance assumption across groups ($p = 0.003$ for yield, and $p < 0.05$ for the other parameters). Therefore, Welch's ANOVA was applied as a robust alternative, followed by Tamhane's post-hoc test, which does not assume equal variances. The results revealed that spawn run time was significantly delayed in the 50OW+50AK group compared to both 100AK and 70OW+30AK ($p = 0.020$ for both). No significant differences were found among the other AK doses or for the remaining parameters, including yield, BE, primordium formation, first harvest, and total harvest time ($p > 0.05$ for all other pairwise comparisons).

According to Table 5, significant differences emerged among the mushrooms produced from the substrates depending on the substrate type. The highest total phenolic content was determined in mushrooms grown on the 100HB substrate with 58.7 mg GAE/g, followed by 100AC, 100AS, and 100WS substrates, respectively. The lowest total phenolic content was found in the 100OW substrate with 19.3 mg GAE/g. The lower phenolic content of mushrooms grown on oak sawdust compared to those grown on hazelnut branches is thought to be related to the high holocellulose and low lignin ratio in oak sawdust (Yıldız *et al*. 2017). *P. ostreatus* did not secrete enzymes to produce phenolic secondary metabolites in 100OW, which has a low lignin content. However, it is thought that substrates with a balanced holocellulose-to-lignin ratio, such as 100HB, require a greater need for enzyme secretion (Akcay *et al*. 2023). Because high total phenolic content is directly related to antioxidant capacity, this should also be taken into consideration in substrate selection.

When total nitrogen and protein contents were examined, nitrogen and protein amounts varied according to the cultivation substrates. The highest nitrogen and protein contents were determined in the 100OW substrate (6.67 g/100 g and 29.21 g/100 g, respectively), followed by 100AK, 100AB, 100WS, 100AS, and 100HB, respectively. The total phenolic contents and protein contents of the mushrooms differed. While the phenolic content was highest in mushrooms grown on hazelnut branches, the lowest protein value was determined in this substrate. The protein value of mushrooms grown on oak acorn kernel (100AK) ranked second with 28.43 g/100 g. It was observed that the different structural characteristics of the substrates used in the study caused variations in protein and phenolic content.

Substrates with high nitrogen and protein content, such as oak sawdust (100OW) and oak acorn kernel (100AK), provided a rich nutrient matrix for mushroom mycelia, directing cells toward primary metabolism (vegetative growth). However, it is thought that this protein-rich "comfortable" environment did not stimulate the survival and defense mechanisms of the mushroom, thereby suppressing the synthesis pathways of phenolic compounds, as reflected by the total phenolic content (expressed as GAE) (Bellettini *et al*. 2019). Furthermore, it is known that the natural phenolic content and bioactive component profiles of the substrates also affect the phenolic content of the mushrooms. The literature indicates that hazelnut wastes are also rich in various polyphenols (Bottone *et al*. 2019). Because the HB material was degraded by the mushroom, it may have incorporated phenolic components into its structure, resulting in mushroom caps rich in phenolic compounds.

Although oak wood also contains phenolic components, the more complex structure of these phenolic compounds in this substrate may have made it difficult for the mushrooms to absorb them (Strapáč *et al*. 2017). In mushrooms grown on the 100AK substrate, both protein and total phenolic contents were found to be higher than those grown on other substrates. This value indicates that the 100AK substrate provided a suitable cultivation medium for both protein and total phenolic content. It is thought that the hot water pasteurization of the 100AK substrate prior to cultivation may have facilitated the uptake of polyphenols by *P. ostreatus* mycelia (Antony and Farid 2022; Ložienė and Petraitytė 2026).

Table 5. The Amount of Total Nitrogen, Protein and Phenolic Content of the Cultivated Mushrooms

Substrates	Total Nitrogen (g/100 g)	Protein (g/100 g)	Total Phenolic Content (mg GAE/g)
100HB	3.61	15.81	58.7 (0.00) *a*
100WS	4.48	19.62	34.8 (0.00) *b*
100AB	4.94	21.64	20.4 (0.00) *c*
100OW	6.67	29.21	19.3 (0.00) *d*
100AK	6.49	28.43	46.3 (0.00) *e*
100AS	4.14	18.13	39.0 (0.00) *f*

The values in parentheses are standard values. Same letters in the same column indicate no significant difference ($p < 0.05$).

The mineral contents of the mushrooms varied significantly according to the cultivation substrates ($p < 0.05$) (Table 6).

Table 6. Elemental Analysis of Mushrooms by Substrate Type

Sub-strates	Elements (mg/kg)							
	Ca	Cu	Fe	Mn	P	Na	Zn	Mg
100HB	106.0 (0.28) *c*	nd	22.3 (2.85) *b*	2.8 (0.38) *d*	1.8 (0.14) *b*	16.6 (8.23) *d*	19.6 (2.99) *c*	0.8 (0.05) *d*
100WS	100.6 (0.33) *c*	7.1 (0.67) *c*	15.9 (1.03) *d*	3.8 (0.27) *c*	2.0 (0.14) *b*	43.8 (6.79) *c*	18.2 (1.43) *d*	1.0 (0.04) *a*
100AB	121.6 (0.91) *b*	0.6 (0.04) *e*	21.2 (1.43) *b*	2.5 (0.16) *e*	1.7 (0.13) *c*	52.1 (3.07) *b*	19.3 (0.61) *c*	0.8 (0.01) *d*
100OW	49.4 (0.41) *e*	18.5 (1.1) *a*	19.8 (1.05) *c*	4.6 (0.11) *a*	3.1 (0.10) *a*	65.6 (4.03) *a*	25.4 (0.23) *a*	0.8 (0.03) *c*
100AK	59.0 (0.35) *d*	16.8 (0.3) *b*	17.4 (0.40) *d*	4.4 (0.11) *b*	3.2 (0.30) *a*	41.0 (1.13) *c*	20.9 (2.02) *b*	0.9 (0.04) *b*
100AS	130.6 (2.24) *a*	2.3 (0.6) *d*	36.4 (2.04) *a*	2.7 (0.15) *d*	2.0 (0.11) *b*	51.7 (4.52) *b*	20.6 (1.85) *b*	1.0 (0.02) *a*

The values in parentheses are standart values, Same letters in the same column indicate no significant difference ($p < 0.05$), nd; not detected

The mineral contents of mushrooms grown on oak sawdust and oak acorn substrates were generally found to be higher compared to those grown on other substrates. Cu, Mn, P, Na, and Zn elements were obtained at higher values in mushrooms grown on oak sawdust and oak acorn wastes compared to other substrates. In terms of Ca, the highest value was determined in the 100AS substrate with 130 mg/kg. For Cu, the highest values were determined in 100OW and 100AK substrates with 18.5 mg/kg and 16.8 mg/kg, respectively, and these were statistically significantly different from the values of mushrooms grown on other substrates. In terms of Fe, a low value (17.4 mg/kg) was determined in the 100AK substrate. When the Mn content of mushrooms grown on the substrates was examined, the highest values were determined as 100OW (4.6 mg/kg) and 100AK (4.4 mg/kg); for P, 100OW (3.1 mg/kg) and 100AK (3.2 mg/kg); and for Zn, 100OW (25.4 mg/kg) and 100AK (20.9 mg/kg). The higher mineral contents observed in mushrooms grown on oak sawdust (100OW) and oak acorn kernel (100AK) may be related

to the natural tendency of oak trees to accumulate minerals from the soil through their deep root systems, and the storage of these elements in acorns to support embryonic development, nucleic acid synthesis, and enzyme activation (Nikolić *et al*. 2006). However, since the present work did not include analysis of the elemental composition of the raw substrates or calculate bioaccumulation factors, this interpretation remains to be confirmed. Future studies should measure substrate mineral content and calculate bioaccumulation factors to verify this hypothesis (Golian *et al.* 2022).

Economic analysis results of the substrates are presented in Table 7. According to the table, the substrate-based benefit–cost ratio (B:C) —considering only substrate procurement and pretreatment costs— was highest in the 50OW+50AK mixture with 4.98, followed closely by 100CC (4.93). However, the difference between these two values is marginal, and no statistical uncertainty was estimated for the economic parameters. In terms of net profit, the highest value (73.56 ₺) was obtained from 50WS+50CC, while 50OW+50AK yielded 51.75 ₺.

Table 7. Economic Analysis Results of Mushrooms Cultivated on Different Substrates (Gross Income, Net Income, and Benefit–Cost Ratio)

Substrates	Mean Yield (g/kg)	Substrates Cost ₺ ($)	Gross income ₺ ($)	Net Income ₺ ($)	Benefit–Cost (B:C) Ratio
100WS	162.8	20.00 (0.43)	73.26 (1.56)	53.26 (1.13)	3.66 (0.08)
HB100	173.8	25.00 (0.53)	78.21 (1.66)	53.21 (1.13)	3.13 (0.07)
100CC	197.1	18.00 (0.38)	88.69 (1.89)	70.69 (1.50)	4.93 (0.10)
100AC	32.5	16.00 (0.34)	14.62 (0.31)	-1.38 (-0.03)	0.91 (0.02)
100AS	31.2	15.00 (0.32)	14.04 (0.30)	-0.96 (-0.02)	0.94 (0.02)
100AB	132.2	24.00 (0.51)	59.49 (1.27)	35.49 (0.76)	2.48 (0.05)
50WS+50CC	205.7	19.00 (0.40)	92.56 (1.97)	73.56 (1.57)	4.87 (0.10)
50WS+50AS	120.0	17.50 (0.37)	54.00 (1.15)	36.50 (0.78)	3.09 (0.07)
50WS+50AC	123.7	18.00 (0.38)	55.66 (1.18)	37.66 (0.80)	3.09 (0.07)
50WS+50HB	125.8	22.50 (0.48)	56.61 (1.20)	34.11 (0.73)	2.52 (0.05)
50WS+50AB	81.3	22.00 (0.47)	36.59 (0.78)	14.59 (0.31)	1.66 (0.04)
100AK	68.7	12.00 (0.26)	30.91 (0.66)	18.91 (0.40)	2.58 (0.05)
100OW	120.6	14.00 (0.30)	54.27 (1.15)	40.27 (0.86)	3.88 (0.08)
90OW+10AK	143.5	13.80 (0.29)	64.57 (1.37)	50.77 (1.08)	4.68 (0.10)
70OW+30AK	119.8	13.40 (0.29)	53.91 (1.15)	40.51 (0.86)	4.02 (0.09)
50OW+50AK	143.9	13.00 (0.28)	64.75 (1.38)	51.75 (1.10)	4.98 (0.11)

Costs include substrate material and, where applicable, AK pretreatment (water, heating, drying, and solid loss). Common costs (spawn, bags, lime, labor, sterilization energy, and overhead) are excluded as they were identical for all treatments. Selling price: 450 ₺/kg (~9.6 USD/kg), based on average retail price from e-commerce platforms, Türkiye (June–August 2026), using the exchange rate on 8 August 2026 (1 USD-$ ≈ 47 ₺). Values in parentheses are expressed in USD.

The rich nutrient content released by hot water pasteurization of the AK material, combined with the low cost of OW sawdust (13.00 ₺), contributed to the favorable B:C ratio of this combination. The combinations of AK material with OW generally gave higher B:C ratios compared to other substrates. For commercial enterprises, the 50OW+50AK and 100CC substrates offer competitive B:C ratios, but net profit should also be considered when selecting a substrate. In contrast, formulations such as 100AC and 100AS, which have low biological efficiency and are not worth the labor and collection costs, should be

avoided. However, it has been determined that these wastes can be economically utilized by mixing them with WS.

In the literature, there are studies on the cultivation of *P. ostreatus* on different lignocellulosic wastes and their economic returns. Thangaraj *et al*. (2026) determined the highest benefit–cost ratio as 2.67 on banana peels. In the present study, the highest benefit–cost ratio obtained in the 50OW+50AK substrate was 4.98, which is 1.86 times higher than the value reported by Thangaraj *et al*. (2026).

The hot water pretreatment applied to oak acorn kernels was assumed to reduce tannin content, but residual phenolic compounds were not quantified. Therefore, the effectiveness of this pretreatment remains unconfirmed. Future studies should include quantitative analysis of tannins before and after pretreatment to optimize the process and assess its impact on mushroom yield and quality.

CONCLUSIONS

1. The use of substrates containing oak acorn kernel (*Quercus ithaburensis* Decaisne) in oyster mushroom (*P. ostreatus*) cultivation was comprehensively investigated for the first time and compared with conventional growing media. The findings obtained in the study revealed that oak acorn kernel (AK) can be successfully used as a substrate component for oyster mushroom production. Furthermore, it was observed that AK material offers both nutritional and economic advantages.
2. Among the substrates used in the study, the highest yield was obtained in 50WS+50CC (205.7 g/kg), while the highest biological efficiency (57.6%) was determined in the 50OW+50AK mixture. The substrate-based B:C ratio was highest for 50OW+50AK (4.98), closely followed by 100CC (4.93); however, the highest net profit (73.56 ₺) was obtained from 50WS+50CC. AK supplementation significantly improved the economic return of OW when compared on a substrate-cost basis
3. The highest protein contents (28.43 to 29.21 g/100 g) were determined in mushrooms grown on AK and OW substrates. The AK was the second-best substrate in terms of protein and phenolic content balance. The highest total phenolic content was determined in the HB material.
4. According to the chemical analyses of the substrates, substrates containing low alpha-cellulose and lignin, such as OW, HB, and WS, provided high yields (120.6 g/kg, 173.8 g/kg, and 162.8 g/kg, respectively), while substrates with high alpha-cellulose content, such as AS and AC, exhibited low yields (31.2 to 32.5 g/kg, respectively).
5. Mineral analyses showed that mushrooms grown on oak-based substrates (OW and AK) had higher Cu, Mn, P, and Zn values, which may be related to the natural mineral accumulation tendency of oak trees. However, since substrate mineral content was not analyzed, this interpretation remains to be confirmed.

6. Oak acorn kernel (AK), which is currently underutilized or left as agricultural waste, represents a valuable alternative substrate for oyster mushroom cultivation. The 50OW+50AK combination showed the highest substrate-based B:C ratio and competitive biological efficiency, although the highest yield and net profit were obtained from 50WS+50CC. Nutritional analyses were not performed for mixed substrates, so no multi-criteria comparison could be made. Future studies should include full nutritional characterization of mixed substrates and apply a formal multi-criteria decision-making framework to objectively evaluate overall substrate.

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Conflict of Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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The authors utilized DeepSeek V4-Pro (https://www.deepseek.com/) for English editing and language refinement of the manuscript.

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