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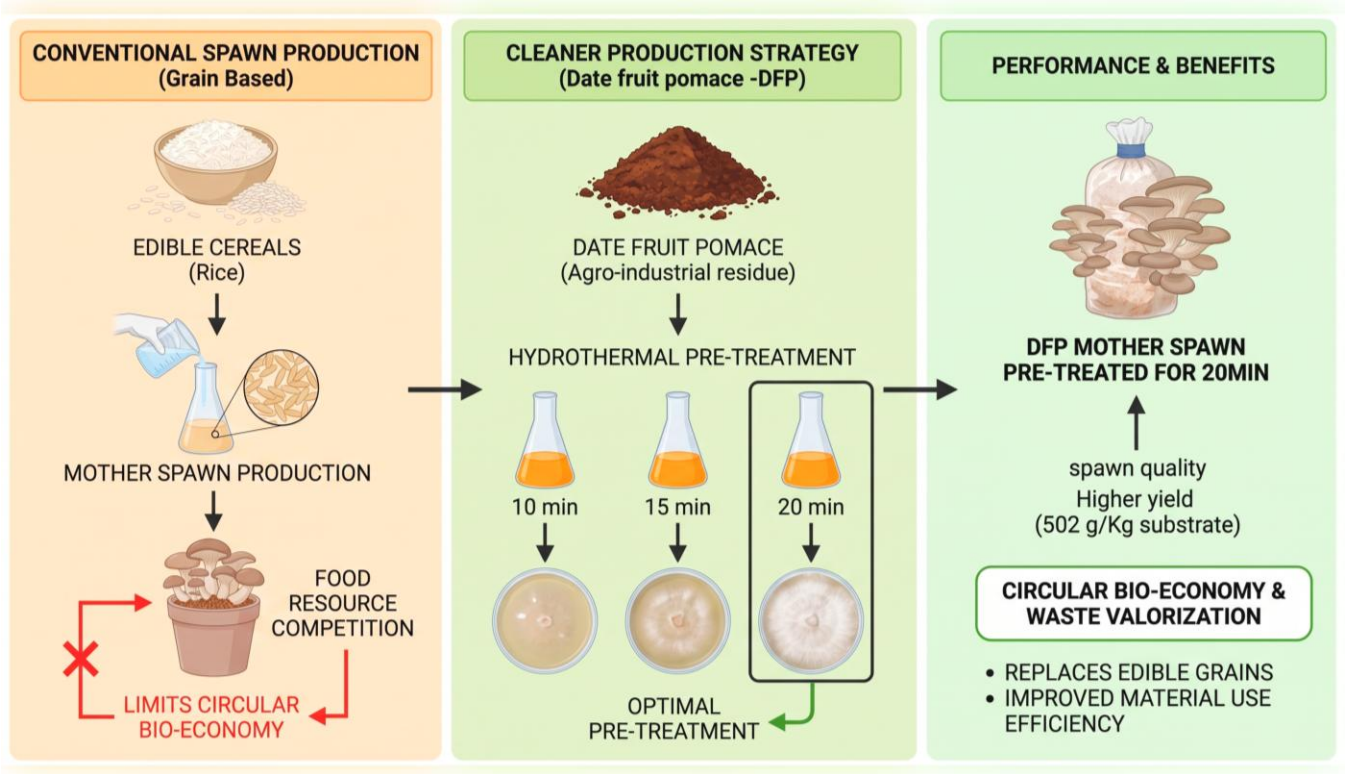
Thermal pre-treatment and biological valorization of date fruit pomace as a grain-free mother-spawn carrier for Elm-oyster mushrooms (*Hypsizygus ulmarius*)

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Abstract

This study evaluated date fruit pomace (DFP), an underutilized date-syrup by-product, as a grain-free mother-spawn carrier for *Hypsizygus ulmarius*, aiming to replace food-grade rice while advancing biomass valorization. DFP was hydrothermally pretreated by boiling for 10, 15, or 20 min (DFP10, DFP15, and DFP20) before mother-spawn preparation. Rice-based spawn served as the control. Fruiting performance, biological efficiency, morphology, elemental composition, protein, phenolics, flavonoids, and antioxidant activity were assessed. Thermal pretreatment significantly influenced spawn performance and mushroom quality. DFP20 produced the highest fresh yield (502.2 g kg^{-1} wet substrate) and biological efficiency (100.4%), representing a 46.2% yield increase over rice spawn. It also increased apparent mycelial biomass (8.93 g versus 5.40 g in the control), protein content (10.80 versus 7.15 g 100 g^{-1} dry weight), total phenolics, and radical-scavenging capacity, although colonization and fruiting were delayed. DFP15 yielded the highest potassium, magnesium, sodium, iron, and zinc concentrations in fruiting bodies. Lead and cadmium remained at low or non-detectable concentrations across treatments.

Appropriately pretreated DFP can replace rice as a mother-spawn carrier and generate high-yielding, nutritionally enhanced elm oyster mushrooms. This approach valorizes date-processing biomass, reduces dependence on edible grains, and supports circular resource use in mushroom production thereby leading to a cleaner food production system.

Keywords

Date fruit pomace; agro-industrial waste valorisation; *Hypsizygus ulmarius*; mother spawn; thermal pretreatment; circular bioeconomy

1. Introduction

Mushroom cultivation is a biologically intensive production system with strong potential to support sustainable food production through its low resource requirements and capacity to valorize lignocellulosic residues (1) (2) . Within this system, inoculum quality (spawn) plays a decisive role in determining productivity, resource efficiency, and economic viability. Mother spawn—the primary generation derived directly from pure culture—forms the foundation for subsequent commercial spawn multiplication and crop establishment (3) . High-quality mother spawn ensures rapid substrate colonization, reduces contamination losses, and improves biological efficiency, thereby directly influencing input-use efficiency and overall system sustainability (4) ; (5) . Conversely, poorly prepared or degenerated spawn can delay crop cycles, increase contamination, and escalate production costs (5). Conventional mother spawn production relies predominantly on cereal grains such as rice and wheat as carrier substrates (6) . While agronomically effective, this practice raises concerns within cleaner production and circular bioeconomy frameworks. Food-grade grains require dedicated agricultural inputs—including land, irrigation, fertilizers, and energy—and their diversion to spawn production may contribute to food-resource competition, particularly in regions facing food security constraints. The continued dependence on edible grains therefore conflicts with strategies aimed at waste valorization and resource substitution in sustainable agri-food systems.

Date fruit pomace (DFP), a fibrous by-product of the date syrup processing industry, represents an abundant and underutilized agro-industrial residue in the Middle East and North Africa. Rich in dietary fiber, residual sugars, and minerals, DFP offers a potentially suitable matrix for fungal colonization while simultaneously providing an opportunity to integrate waste streams into high-value food production (7) ; (8)). The suitability of residue-derived substrates is supported by studies, where substrate composition and cultivation conditions influenced mushroom productivity, morphology, mineral accumulation, vitamin content, phenolic compounds, and antioxidant activity (9) Thus, valorizing DFP as a spawn carrier could (i) displace food-grade grains, (ii) improve material-use efficiency, and (iii) strengthen circular resource flows within regional agri-industrial systems.Valorizing DFP as a spawn carrier could therefore (i) displace food-grade grains, (ii) enhance material-use efficiency, and (iii) strengthen circular resource flows within regional agri-industrial systems.

Previous studies have demonstrated the suitability of date palm residues—such as fronds, pits, and fruit pulp—as fruiting substrates for oyster mushroom (10) . However, the use of date-processing by-products as carrier substrates for mother spawn—the critical upstream stage governing subsequent productivity—remains largely unexplored. Furthermore, the influence of controlled thermal pretreatment on spawn quality, yield performance, nutrient enrichment, and functional properties has not been systematically evaluated.

Edible mushrooms are valuable food materials because they provide protein, amino acids, minerals, vitamins, dietary fiber, and bioactive compounds(11) . Recent research further indicates that mushroom quality is not determined solely by species but can also be modified by cultivation conditions. For example, blue-light exposure altered productivity, morphological traits, total phenolic content, and antioxidant activity in several cultivated mushroom species, including *Pleurotus ostreatus* and *Pleurotus djamor* (12) . Similarly, solid-state fermentation of wheat bran with mushroom cultures modified dietary-fiber fractions and phenolic content, demonstrating the capacity of mushrooms to transform plant by-products into nutritionally improved materials (13) . These findings support the evaluation of DFP not only as a physical carrier for mother spawn but also as a substrate capable of influencing the nutritional and functional quality of the resulting fruiting bodies.

This study therefore investigates thermally pretreated DFP as a grain-free carrier substrate for mother spawn production of *Hypsizygus ulmarius* (Elm oyster mushroom). Specifically, the objectives were to (i) optimize boiling duration to improve sterility and nutrient accessibility, (ii) evaluate production performance and biological efficiency relative to rice-based spawn, and (iii) assess elemental composition, product safety, and antioxidant-related phytochemicals of the resulting fruiting bodies. By integrating agronomic performance with resource-substitution analysis, this study evaluates the feasibility of valorizing date fruit pomace (DFP), an agro-industrial residue, as a cleaner-production input for sustainable mushroom cultivation and biomass-circularity pathways.

2. Materials and Methods

Materials and Methods are provided in Supplementary Information as Online Resource 1.

3. Results and Discussion

3.1 Thermal optimization, mycelial growth, and spawn quality

Boiling duration of date fruit pomace (DFP) markedly influenced its performance as a mother-spawn carrier for *Hypsizygus ulmarius*. Mycelial biomass increased significantly with boiling time ($p = 0.010$), from 5.40 g in the rice control to 8.67 and 8.93 g in DFP15 and DFP20, respectively; both values were significantly higher than the control ($p < 0.05$). However, colonization was fastest in the rice control, requiring 15.33 days, and progressively slower in DFP10, DFP15, and DFP20 ($p = 0.002$). Similarly, the rice control significantly advanced pinning and first harvest, occurring at 19.45 and 25.33 days, respectively, whereas DFP20 required 23.32 and 30.67 days ($p < 0.001$). DFP15 produced fruiting times comparable to the control, while DFP20 imposed a clear developmental delay (Fig. 1).

The longer colonization and fruiting periods observed after DFP pretreatment were accompanied by increased mycelial biomass and reduced contamination. Contamination was highest in DFP10 (30%), followed by DFP15 (20%), whereas the control and DFP20 each recorded 10% contamination. Thus, contamination was 20 and 10 percent higher than the control in DFP10 and DFP15, respectively. These results indicate that boiling duration affected both the sanitary condition and nutritional suitability of the carrier substrate. The progressive reduction in contamination with longer boiling suggests that extended thermal treatment more effectively suppressed competing microorganisms. However, the associated delay in colonization and fruiting indicates that pretreatment intensity also altered substrate structure and nutrient accessibility.

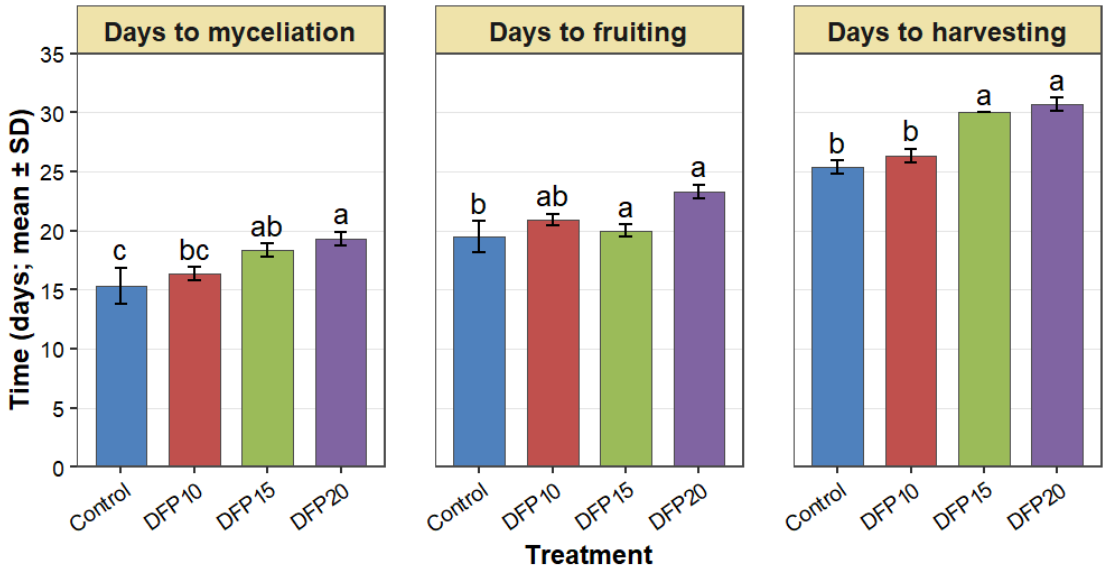


Fig 1. Effect of different DFP treatments on the time required for mycelial colonization, fruiting initiation, and mushroom harvesting. Bars represent mean values ± standard deviation (SD), expressed in days. Different lowercase letters above the bars indicate statistically significant differences among treatments according to Tukey's honestly significant difference (HSD) post-hoc test at $p < 0.05$. Bars sharing at least one common letter are not significantly different.

This pattern agrees with previous studies of hydrothermal pretreatment of lignocellulosic materials. Boiling, autohydrolysis, and liquid-hot-water treatment can modify water chemistry at elevated temperatures, promote partial cleavage of hemicellulosic and amorphous polysaccharide bonds, and increase access to structural carbohydrates (14) (15) ; (16,17) In the present study, moderate boiling for 15 min may have partially depolymerized hemicelluloses and exposed fermentable compounds while retaining sufficient structural integrity for hyphal attachment. It may also have reduced competing microbiota without causing excessive nutrient loss, thereby creating a favorable environment for *H. ulmarius(18)* .

By contrast, the 10-min treatment may have provided insufficient microbial suppression and substrate conditioning, whereas the 20-min treatment increased mycelial biomass and yield at the cost of a longer crop cycle. Excessively severe hydrothermal treatment can over-solubilize readily available carbohydrates, disrupt the substrate matrix, and promote leaching of soluble nutrients (19) . Therefore, thermal pretreatment should be optimized to provide adequate microbial control and improve substrate accessibility without unnecessarily prolonging production or reducing nutrient retention.

3.2 Yield, morphological responses, and biological trade-offs

Fresh mushroom yield differed significantly among treatments ($p < 0.001$). DFP20 produced the highest fresh yield, 502.2 g kg^{-1} wet substrate, representing a 46.2% increase relative to the rice control, which produced 343.5 g kg^{-1}. In contrast, DFP15 and DFP10 produced 260.9 and 250.7 g kg^{-1}, respectively. Biological efficiency followed the same pattern, with DFP20 reaching approximately 100.4% and significantly exceeding the other treatments ($p < 0.05$). Dry mushroom yield did not differ significantly among treatments ($p = 0.448$). These findings indicate that extended boiling improved the conversion of DFP-based spawn into fresh mushroom biomass, although it delayed crop development(Table 1).

Table 1. Yield and morphological characteristics of *Hypsizygus ulmarius* cultivated using different mother-spawn treatments

Parameter	Control	DFP10	DFP15	DFP20	ANOVA *p*-value
Fresh yield (g kg^{-1} substrate)	343.5 ± 24.8^{b}	250.7 ± 64.6^{b}	260.9 ± 20.4^{b}	502.2 ± 39.1^{a}	<0.001
Dry yield (g kg^{-1} substrate)	37.58 ± 3.94^{a}	32.22 ± 4.54^{a}	32.69 ± 3.37^{a}	33.93 ± 4.94^{a}	0.448
Mycelial biomass (g)	5.40 ± 0.95^{c}	5.83 ± 1.27^{bc}	8.67 ± 1.36^{ab}	8.93 ± 1.00^{a}	0.01
Cap diameter (cm)	4.97 ± 1.92^{a}	6.58 ± 1.59^{a}	4.85 ± 2.55^{a}	4.42 ± 1.02^{a}	0.227
Stipe length (cm)	5.32 ± 0.84^{a}	3.28 ± 1.95^{ab}	2.38 ± 1.45^{b}	2.17 ± 0.46^{b}	0.002
Cap diameter/stipe length ratio	0.97 ± 0.45^{b}	2.55 ± 1.42^{a}	2.34 ± 0.89^{ab}	2.08 ± 0.56^{ab}	0.032

Values are presented as mean ± SD. Different superscript letters within a row indicate significant differences according to Tukey's HSD test ($p \leq 0.05$). DFP10, DFP15, and DFP20 represent date fruit pomace boiled for 10, 15, and 20 min, respectively.

The yield response is consistent with reports that boiling time influences substrate softness, moisture content, microbial load, and nutrient availability, thereby affecting biological efficiency and productivity in oyster mushroom cultivation; (20,21)). Boiling sorghum grains for 30 min improved the quality and colonization of *Pleurotus ostreatus* mother spawn, while moderate boiling of sugarcane leaves accelerated pinning and reduced crop duration; longer boiling periods increased flush number and total yield (20,21) .Similarly, DFP20 generated the highest yield and biological efficiency in the present study. However, this increase was coupled with delayed fruiting and lower mineral density, suggesting that extended boiling may promote biomass accumulation while also causing nutrient dilution or heat-induced leaching.

Cap diameter did not differ significantly among treatments ($p = 0.227$), with mean values ranging from 4.42 cm in DFP20 to 6.58 cm in DFP10. In contrast, stipe length differed significantly ($p = 0.002$). The rice control produced the longest stipes (5.32 cm), followed by DFP10 (3.28 cm), DFP15 (2.38 cm), and DFP20 (2.17 cm). Stipe length therefore decreased with increasing boiling duration, and the values recorded for DFP15 and DFP20 were significantly lower than those of the control according to Tukey's

HSD test ($p < 0.05$). The cap diameter-to-stipe length ratio also varied significantly ($p = 0.032$), increasing from 0.97 in the control to 2.55 in DFP10, with intermediate values in DFP15 and DFP20. These results indicate that DFP-based spawn promoted proportionally broader caps and shorter stipes (Table 1).

The combined growth and yield response demonstrates a biological trade-off between rapid crop establishment, total biomass production, and nutritional quality. Although DFP15 maintained fruiting times closer to the control and maximized mineral enrichment, DFP20 produced the greatest fresh yield and mycelial biomass. Similar trade-offs have been reported for cereal- and residue-based substrates, where longer boiling improves cumulative yield but moderate boiling better preserves nutrient content and supports more balanced crop development (20,21). Thus, DFP20 appears optimal when fresh yield and biological efficiency are the primary objectives, whereas DFP15 may be preferable when shorter production time and mineral density are more important.

Overall, boiling DFP for 20 min provided the most favorable balance between substrate sanitation, mycelial biomass, and fresh mushroom yield, despite extending the colonization and fruiting periods. These results confirm that DFP can function as a grain-free mother-spawn carrier for *H. ulmarius*, but the optimum boiling duration should be selected according to the intended production objective.

3.3 Nutritional uptake, safety, and yield–quality trade-offs

Elemental analysis by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP–OES)showed that thermal pretreatment of date fruit pomace (DFP) significantly altered mineral accumulation in *Hypsizygus ulmarius* fruiting bodies relative to raw DFP and the rice control (Table 2). Among the macronutrients, potassium (K), magnesium (Mg), and sodium (Na) were strongly enriched in the mushrooms compared with raw DFP. Potassium reached its highest concentration in DFP15 (29.16 mg g^{-1} DW), followed by DFP10 (20.76 mg g^{-1} DW), the rice control (17.46 mg g^{-1} DW), and DFP20 (16.77 mg g^{-1} DW), whereas raw DFP contained only 3.96 mg g^{-1} DW. Magnesium and sodium showed similar patterns, with maximum concentrations in DFP15 at 1.89 and 0.59 mg g^{-1} DW, respectively. In contrast, calcium (Ca) decreased relative to raw DFP and reached its lowest concentration in DFP20 (0.46 mg g^{-1} DW), indicating more limited Ca transfer under prolonged boiling.

The selective accumulation of minerals indicates that uptake was not proportional to their concentrations in the original substrate. Raw DFP provided a mineral-rich starting matrix, particularly for K, Mg, and Ca; however, following cultivation, K, Mg, and Na were substantially enriched in the fruiting bodies, whereas Ca was consistently reduced. This pattern agrees with previous observations that *Hypsizygus* and related mushroom species preferentially accumulate K and selected micronutrients, while Ca uptake is comparatively restricted (22) . Moderate boiling may have disrupted mineral–organic complexes and increased mineral solubilization, thereby improving the availability of K, Mg, and Na for fungal uptake. The lower Ca concentrations observed after cultivation, particularly in DFP20, may reflect limited physiological transfer or calcium losses during extended thermal treatment.

For micronutrients, the rice control exhibited the highest Fe and Zn concentrations, at 0.157 and 0.0741 mg g^{-1} DW, respectively. DFP15 showed the next highest concentrations, with 0.106 mg g^{-1} DW Fe and 0.0685 mg g^{-1} DW Zn. The decline in Fe and Zn concentrations under DFP20, despite its higher mushroom yield, suggests that extended boiling may have caused mineral leaching or dilution through rapid biomass accumulation. The Fe response is consistent with the dual role of iron in *Pleurotus* species, where iron is essential for metabolism but excessive concentrations may suppress mycelial growth and biomass production (23) . In the present study, Fe enrichment under DFP15 remained compatible with vigorous growth, whereas the lower Fe concentration in DFP20 appears more likely to reflect process-related losses than physiological limitation.

Zinc showed a comparable response. Poursaeid et al. (2015) (24) reported that zinc-enriched media increased biomass production and Zn accumulation in *Pleurotus florida*, with uptake closely associated with fungal growth rather than passive accumulation. In the present study, Zn increased substantially from raw DFP to mushroom tissue and reached its highest concentration under DFP15. This finding suggests that moderate pretreatment created a favorable window for Zn mobilization and assimilation. The subsequent reduction in Zn concentration under DFP20 indicates that prolonged thermal exposure may compromise Zn retention in the substrate even when overall mushroom yield is increased.

Heavy-metal concentrations remained low across all treatments. Lead (Pb) ranged from 0.0005 to 0.0017 mg g^{-1} DW, while Cd was below detection or remained well below the applicable Codex and WHO safety limits(25) . These results support the safety of DFP-based substrates for mushroom production. Nevertheless, heavy-metal safety should continue to be verified when DFP is obtained from different processing facilities because the elemental composition of agro-industrial residues may vary according to soil, irrigation water, processing equipment, and storage conditions.

When yield and mineral data were considered jointly, DFP20 produced the highest fresh yield, 502.2 g kg^{-1} substrate, but the lowest total mineral density, 18.55 mg g^{-1} DW. In contrast, DFP15 produced the highest total mineral content, 32.01 mg g^{-1} DW, while achieving a moderate fresh yield of 260.9 g kg^{-1} substrate. Thus, DFP20 was advantageous for maximizing biomass production, whereas DFP15 provided a more favorable nutritional profile. This contrast indicates a yield–quality trade-off in which increased production may dilute the concentration of certain minerals in the fruiting bodies.

Table 2. Comparative elemental composition of raw DFP and *Hypsizygus ulmarius* fruiting bodies produced using rice and DFP-based mother spawn, expressed as mg g^{-1} dry weight.

Element	Raw DFP	Control	DFP 10 min	DFP 15 min	DFP 20 min
K	3.96 ± 0.12^{e}	17.46 ± 0.04^{c}	20.76 ± 0.11^{b}	29.16 ± 0.19^{a}	16.77 ± 0.12^{d}
Mg	0.68 ± 0.02^{e}	1.57 ± 0.01^{b}	1.48 ± 0.00^{c}	1.89 ± 0.01^{a}	1.22 ± 0.01^{d}
Ca	1.43 ± 0.06^{a}	0.80 ± 0.01^{b}	0.73 ± 0.00^{c}	0.79 ± 0.01^{bc}	0.46 ± 0.00^{d}
Na	0.26 ± 0.01^{e}	0.36 ± 0.00^{d}	0.43 ± 0.00^{b}	0.59 ± 0.01^{a}	0.38 ± 0.00^{c}
Fe	0.045 ± 0.002^{e}	0.157 ± 0.001^{a}	0.077 ± 0.000^{c}	0.106 ± 0.001^{b}	0.048 ± 0.000^{d}
Zn	0.0166 ± 0.0006^{e}	0.0741 ± 0.0006^{a}	0.0630 ± 0.0002^{c}	0.0685 ± 0.0004^{b}	0.0469 ± 0.0002^{d}
Pb	0.00125 ± 0.00048^{a}	0.00170 ± 0.00100^{a}	0.00050 ± 0.00043^{a}	0.00170 ± 0.00102^{a}	0.00070 ± 0.00109^{a}

3.4 Correlations between yield and mineral concentrations

Pearson correlation analysis across the four treatment means revealed predominantly positive associations among the mineral concentrations (Fig. 8). The strongest correlations occurred between K and Na (r = 0.99), Zn and Ca (r = 0.98), and Mg and Cu (r = 0.95). Strong positive associations were also observed

between Cu and Ca (r = 0.93) and between Fe and Zn (r = 0.90). These relationships suggest that some minerals may have responded to common substrate- or uptake-related factors during fruiting-body development.

Dry yield was positively correlated with Fe concentration (r = 0.72) but negatively correlated with Na (r = −0.61), K (r = −0.54), and mycelial yield (r = −0.45). Mycelial yield showed negative associations with Zn (r = −0.59), Fe (r = −0.58), and Ca (r = −0.57), but positive associations with Na (r = 0.49) and K (r = 0.36). Its association with Mg was negligible (r = −0.01). Because these correlations were calculated from only four treatment means and statistical significance was not assessed, they should be interpreted as exploratory rather than causal. Nevertheless, the patterns reinforce the conclusion that mineral accumulation in *H. ulmarius* was governed by both substrate composition and species-specific physiological preferences. (Fig 2)



Fig.2 Pearson correlation heatmap of dry yield, mycelial yield, and mineral concentrations across treatment means. Cell values represent Pearson correlation coefficients (r), with red indicating positive correlations and blue indicating negative correlations.

Collectively, the results and previous reports indicate that DFP can serve as an effective mineral source for *H. ulmarius* when thermal pretreatment is appropriately controlled (24) (23) (26) . In particular, the 15-min treatment promoted the greatest accumulation of K, Mg, Na, Fe, and Zn, whereas the 20-min treatment maximized fresh yield but reduced total mineral density. Therefore, DFP15 may be preferable when nutritional enrichment is prioritized, while DFP20 may be selected when maximum mushroom productivity is the primary objective.

3.5 Biochemical characterization of protein and antioxidant-related phytochemicals

Extractable protein content in wheat-substrate-grown elm oyster mushrooms was determined using the Bradford assay at 595 nm and expressed on a dry-weight basis. Protein content was 7.15 ± 0.08 g 100 g^{-1} in the rice control and increased to 8.65 ± 1.51, 9.16 ± 0.25, and 10.80 ± 0.40 g 100 g^{-1} in DFP10, DFP15, and DFP20, respectively (mean ± SD, n = 3). The highest mean protein content was therefore observed in DFP20. These values represent protein recovered under the extraction conditions used and should not be interpreted as total crude protein.

Antioxidant activity, assessed using DPPH and ABTS radical-scavenging assays, also improved with longer boiling durations. DFP20 extracts exhibited the lowest IC_{50} values, indicating the strongest radical-scavenging activity, whereas DFP15 showed the highest IC_{50} values and therefore the weakest activity.

Overall, mushrooms produced using DFP-based mother spawn generally showed greater antioxidant activity than the rice control, particularly after 20 min of boiling.

Total phenolic content (TPC) differed significantly among treatments ($p = 0.0013$). DFP10 had the lowest TPC, at 12.80 mg GAE g^{-1} DW, whereas DFP15, DFP20, and the rice control recorded 14.30, 15.66, and 15.06 mg GAE g^{-1} DW, respectively. These latter treatments formed a statistically similar higher-phenolic group. Thus, short boiling appeared to reduce phenolic accumulation, whereas longer pretreatment restored or enhanced the phenolic content of the fruiting bodies.

Total flavonoid content (TFC) also differed among treatments ($p = 0.0333$). The rice control recorded the highest TFC, at 14.10 mg QE g^{-1} DW, while DFP20 showed the lowest value, at 12.69 mg QE g^{-1} DW. The TFC of DFP20 was significantly lower than that of the control but did not differ significantly from DFP10 or DFP15. Therefore, the 20-min treatment produced a slight reduction in flavonoid content despite increasing TPC and antioxidant activity.

The biochemical responses (Fig 3) suggests that the effect of thermal pretreatment was compound-specific. DFP20 increased extractable protein, phenolic content, and radical-scavenging activity, whereas flavonoid content decreased slightly. This indicates that extended boiling may have enhanced the availability or bioconversion of phenolic precursors without producing a corresponding increase in all flavonoid compounds. Because DPPH and ABTS IC_{50} values were lowest in DFP20, the increase in antioxidant activity appears to be more closely associated with phenolic accumulation and other antioxidant constituents than with total flavonoid concentration.

These results are consistent with reports that the biochemical profile and antioxidant capacity of edible mushrooms are strongly influenced by substrate composition and processing conditions(27) (28) . Similarly, changes in sugarcane-based substrates supplemented with wheat bran altered yield, antioxidant activity, and the production of bioactive compounds, including vitamin E, phenolics, fatty acids, and terpenoids, in *Pleurotus ostreatus* (29) . The improved antioxidant activity observed in the present study may therefore result from the release of bound phenolics and other antioxidant-active constituents from the DFP lignocellulosic matrix during moderate-to-extended boiling. These compounds may subsequently have been absorbed, transformed, or bioconverted during fungal development.

Substrate-mediated changes in antioxidant activity have also been reported in other *Pleurotus* species. Gąsecka et al. (2016) (9) showed that selenium and zinc supplementation increased phenolic compounds, ascorbic acid, and DPPH-scavenging activity in *P. ostreatus* and *P. eryngii*. The authors also reported a relationship between total phenolic content and antioxidant activity, supporting the interpretation that the higher phenolic content observed in DFP20 contributed to its lower DPPH and ABTS IC_{50} values. Similarly, Ahmed et al. (2024) (30) found that waste-tea-leaf supplementation altered the yield, biological efficiency, mineral composition, amino-acid profile, and nutritional quality of *P. ostreatus*. These findings support the view that agro-industrial residues can act not only as structural substrates but also as sources of compounds that influence fungal metabolism and fruiting-body quality.

The findings are also consistent with broader studies of oyster mushrooms. Sultana et al. (2024)(31) reported high levels of phenolics, flavonoids, tannins, and mineral elements, together with strong DPPH-scavenging activity, among eight *Pleurotus* species cultivated in Bangladesh. Their study also confirmed low fat content and heavy-metal concentrations below safety thresholds. More recently, Atila et al. (2026) (32) demonstrated that different residue-based substrate supplements produced distinct changes in the nutritional, mineral, phenolic-related, and antioxidant profiles of *Hypsizygus ulmarius*. In the present study, thermally conditioned DFP similarly supported the production of mushrooms with enhanced phenolic-associated antioxidant activity while maintaining low heavy-metal concentrations.In the present

study, thermally conditioned DFP similarly supported the production of mushrooms with enhanced phenolic-associated antioxidant activity while maintaining low heavy-metal concentrations.

Taken together, the results demonstrate that the processing of DFP as a mother-spawn carrier can influence both the nutritional and functional quality of *H. ulmarius* fruiting bodies. DFP20 produced the highest protein concentration, greatest phenolic accumulation, and strongest antioxidant activity, although it caused a minor reduction in flavonoid content. DFP15 provided a more balanced mineral profile and moderate yield. Thus, DFP20 may be preferred when antioxidant capacity and protein recovery are the main objectives, whereas DFP15 may be more suitable when nutritional balance and mineral enrichment are prioritized.

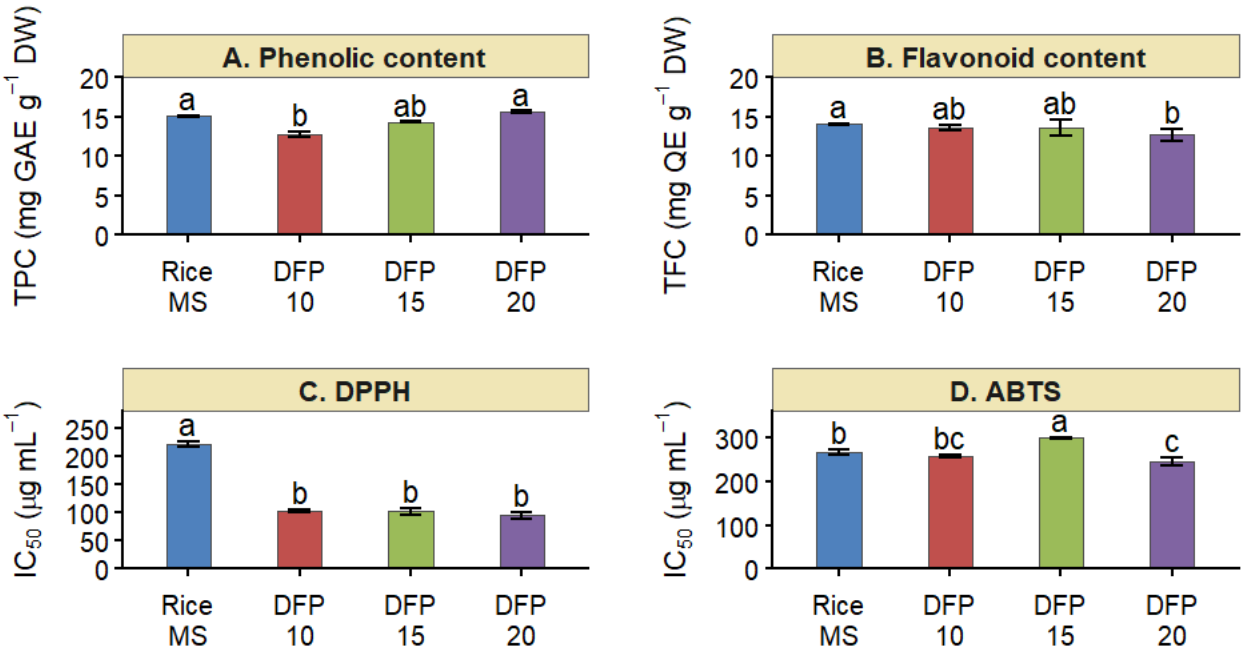


Fig 3. Total phenolic content (A), total flavonoid content (B), DPPH IC_{50} (C), and ABTS IC_{50} (D) of mushrooms produced using rice mother spawn and DFP-based mother spawn prepared with 10, 15, or 20 min of boiling. Bars represent means, and error bars indicate standard deviations. Within each panel, treatments sharing at least one lowercase letter do not differ significantly according to Tukey's HSD test ($\alpha = 0.05$). Lower IC_{50} values indicate stronger antioxidant activity. GAE, gallic acid equivalents; QE, quercetin equivalents; DW, dry weight.

3.6 Multivariate structure of growth and yield (PCA Analysis)

Principal component analysis (PCA) was performed on eight traits, namely cap diameter, stipe length, CD/SL ratio, days to myceliation, days to fruiting, fresh yield per kg substrate, dry yield per kg substrate, and mycelial biomass in order to summarize the multivariate response of the treatments. The first three principal components explained 86.6% of the total variation, with PC1 accounting for 50.6%, PC2 for 20.0%, and PC3 for 16.0%. Thus, the first two components together captured 70.6% of the total variance and provided a reliable representation of treatment separation.

PC1 was positively associated mainly with days to myceliation, days to fruiting, mycelial biomass, and to a lesser extent fresh yield/kg substrate, while it was negatively associated with stipe length, dry yield/kg substrate, and cap diameter. This component can therefore be interpreted as a development–productivity

axis, separating treatments with longer crop duration and greater biomass accumulation from those showing faster crop establishment and stronger stipe expression. PC2 was driven largely by positive loadings for CD/SL ratio and cap diameter, with negative contributions from fresh yield/kg substrate, mycelial biomass, and stipe length, indicating a morphology axis that contrasts cap-related traits with yield-associated traits. PC3, although not shown in the biplot, contributed an additional 16.0% of the variance and was influenced mainly by fresh and dry yield, suggesting a secondary yield-intensity dimension.

In the PC1-PC2 biplot (Fig 4), the control treatment clustered on the negative side of PC1 and PC2, indicating association with shorter crop duration, longer stipe length, and comparatively lower expression of the yield-related variables positioned on the positive PC1 side. DFP10 was separated mainly along positive PC2 and negative PC1, reflecting stronger association with cap diameter and CD/SL ratio, and therefore a more morphology-oriented response. DFP15 occupied an intermediate-to-positive position on PC1 with a relatively broad ellipse, suggesting greater within-treatment variability and a mixed response involving both morphological and yield-related traits. DFP 20 formed a tighter cluster on the positive side of PC1 and slightly negative PC2, indicating a stronger association with mycelial biomass and fresh yield/kg substrate, but also with longer myceliation and fruiting periods. Overall, the treatments followed a gradient from control to DFP20 along PC1, suggesting a progressive shift from a stipe- and rapid-development profile toward a more biomass- and fresh-yield-oriented phenotype.

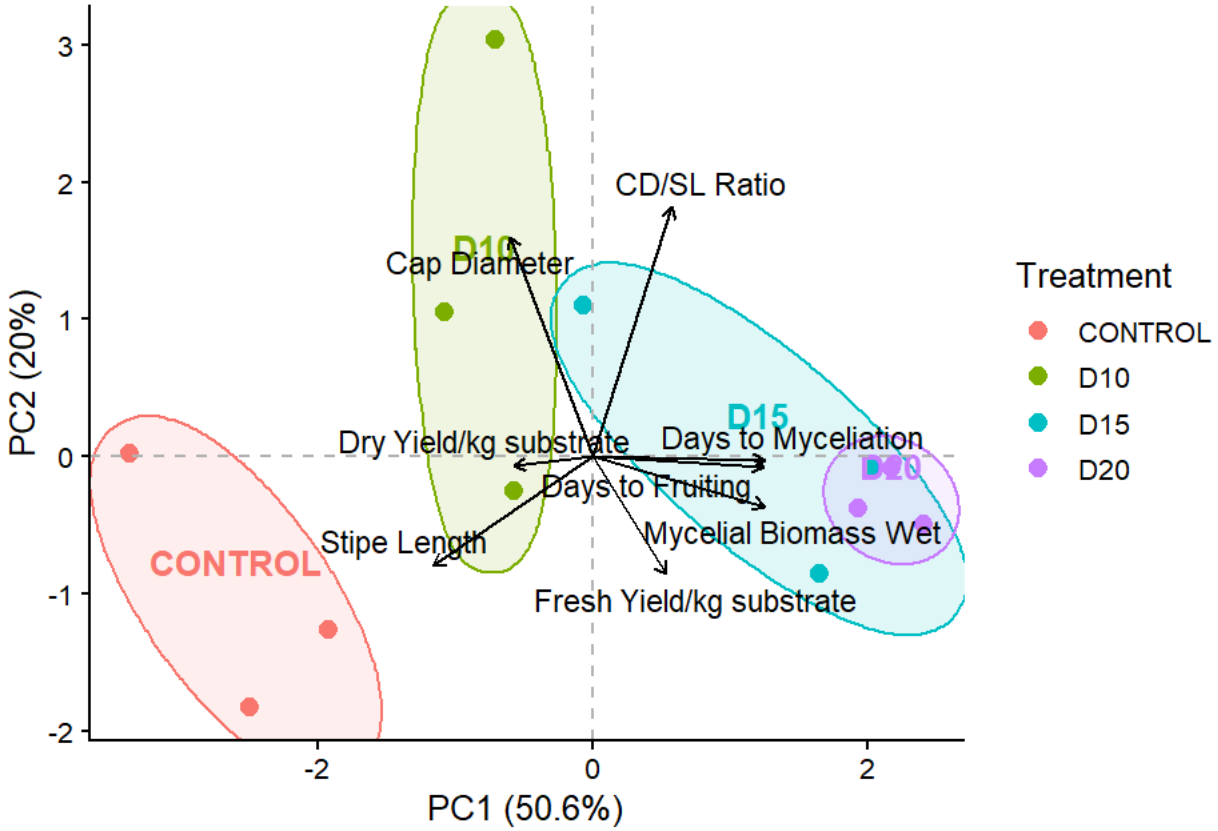


Fig 4. Principal component analysis (PCA) biplot of mushroom growth and yield traits across treatments, where D10, D15, and D20 denote DFP10, DFP15, and DFP20, respectively.PCA biplot shows the multivariate relationships among growth, morphology, and yield traits across treatments. PC1

explained 50.6% and PC2 explained 20.0% of the total variance. Arrows represent trait loadings, while ellipses indicate the clustering pattern of treatment replicates.

Similar multivariate patterns have been reported in mushroom cultivation studies using alternative substrates and pretreatment strategies(33–35) . Atila et al. (2026) (32) used PCA to integrate mineral, amino-acid, fatty-acid, proximate, and antioxidant variables in *Hypsizygus ulmarius*. Their first two principal components explained 62.5% of the total variation and separated mushrooms according to residue-specific substrate supplementation. Ogunsade et al. (2026)(36) also applied PCA to pretreated *H. ulmarius* and showed that antioxidant, proximate, antinutritional, and functional variables contributed to treatment separation. In *Pleurotus ostreatus*, tea-waste substrate formulations produced distinct multivariate patterns based on biological efficiency, mineral composition, amino-acid profile, and nutritional traits (30) . These findings are consistent with the present study, in which PC1 represented a development–productivity gradient, whereas PC2 represented a morphology-related gradient. The separation of the control, DFP10, DFP15, and DFP20 treatments therefore suggests that thermal pretreatment progressively shifted the crop from a rapid-development and stipe-oriented phenotype toward a biomass- and fresh-yield-oriented phenotype.

4. Conclusion

This study demonstrates that date fruit pomace (DFP), an agro-industrial by-product of the date syrup industry, can be successfully valorized as a grain-free carrier substrate for mother spawn production of *Hypsizygus ulmarius*. Controlled thermal pretreatment was essential, as unprocessed DFP showed high contamination, whereas boiling improved sterility, mycelial vigor, and yield performance. Among the tested conditions, 20-minute pretreatment achieved the highest fresh yield (≈502 g kg^{-1} substrate), biological efficiency (>100%), and lowest contamination rate, while 15-minute pretreatment maximized mineral enrichment (K, Mg, Fe, Zn), highlighting a clear productivity–nutrient trade-off.

Elemental profiling confirmed enhanced uptake of essential macro- and micronutrients without exceeding international safety limits for heavy metals. Thermal conditioning also improved protein content and antioxidant activity, indicating that spawn substrate selection can influence nutraceutical attributes of the final product.

From a waste-valorization perspective, DFP-based mother spawn offers a practical route to upcycle an underutilized date-processing residue into a functional input for mushroom production. Its use can reduce reliance on food-grade rice while improving the cascading use of biomass within regional agri-industrial systems. Under optimized pre-treatment, DFP can support efficient resource recovery and contribute to circular bioeconomy models that convert waste streams into high-value food products. Although treatment-specific water and energy demands were not assessed in this study, future life-cycle assessment and techno-economic analysis should quantify the environmental and economic performance of DFP valorization at commercial scale.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions: Conceptualization, SK; methodology, MK; formal analysis, MK;PPT investigation, MK; YKA; resources, SK; data curation, MK and DF; writing—original draft preparation, MK and DF; writing—review and editing, SK and WAM ; visualization, MK and DF; supervision,SK; project administration, SK; funding acquisition, SK. All authors have read and agreed to the published version of the manuscript

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