# Transformation of Olive Mill Stone Waste, Walnut Shell and their mixtures into proteinaceous animal feed via solid state fermentation

Christos Eliopoulos[1], Giorgos Markou[1], Ioanna Langousi[1], Eleni Kougia[1], Patra Sourri[1], Anastasia Liatiri[1], Dimitrios Arapoglou[1*]
[1]Institute of Technology of Agricultural Products, HAO-DEMETER, Athens, Sof. Venizelou 1, 14123, Greece.
*Presenting/Corresponding author email: dimarap@elgo.gr tel: +302102845940
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## Introduction

Food and Agricultural industries produce annually large quantities of wastes and by-products, which are usually disposed into nearby open fields, enhancing environmental pollution which is associated with negative impacts on human and animal health (Eliopoulos et al., 2022). The European Union has focused on the implementation of the circular economy by applying wastes' biotransformation targeting to their depletion. The nutritional content of the agro-industrial wastes is mainly composed by cellulose, hemicellulose, lignin, proteins, carbohydrates, polyphenols, sugars and minerals, which justifies their characterization as raw materials instead of wastes. A lot of endeavors have been carried out for the upgrade and exploitation of these raw materials, in order to indicate their nutritional perspectives, by applying solid-state fermentation (SSF) process. *Pleurotus ostreatus* is classified as an edible white rot fungus, well known as a potent mean for lignin degradation by secreting extracellular hydrolytic and oxidative enzymes (Han et al., 2020).

Mediterranean basin countries such as Greece, Spain, Italy, etc. are the main producers of olive oil. Olive oil production is performed by using a two or three-phase extraction systems where a lot of wastes are derived. Olive press cake and a liquid effluent with dark color which is referred as wastewater, are the main wastes derived from a three phase system. These wastes are mainly disposed in soils and rivers and are considered as toxic due to their high concentration in polyphenolic content, contributing to the environmental pollution (Eliopoulos et al., 2022).

Walnuts are produced from *Juglans regia* L, which is known as the walnut tree. Walnut production is the second largest nut-production after almond nuts, since their production exceeded 3.7 million tons worldwide in 2019. Walnut Shells (WS) form the 67% of fruit's total weight and they are mainly consisted of cellulose, hemicellulose and lignin. These agricultural wastes are usually discarded with no further use post cultivation or incinerated for heating purposes (Albatrni et al., 2022).

Common feature of latter residues is their poor nutritional value, which forms the major inhibitory factor for their valorization in livestock feeding. The goal of this study is the exploitation of Olive Mill Stone Waste (OMSW) and WS along with their mixtures at various ratios, into a novel and nutritional proteinaceous animal feed, through solid-state fermentation process initiated by *P. ostreatus*.

## Materials and Methods

OMSW, WS and their prepared mixtures were used as substrates for the growth of *P. ostreatus*. The optimum moisture was achieved after the addition of tap water to all substrates, which was and renewed every day. The initial dry substrate was milled to a particle size of 2mm. The hydrated samples of OMSW, WS, as well as the addition of WS at 20%,40%,60% and 80%w/w proportions to OMSW, were weighed and mixed to a final weight of 300g. The fermentation process was performed into closed glass test vessels of 750 mL volume. The prepared samples were sterilized by heating at 121 °C for 15min and then were inoculated in a vertical laminar flow chamber by adding *P. ostreatus* strain on their surface at a ratio of 3% w/w. Incubation was carried out in a bioclimatic chamber with a stable temperature at 25 °C in the absence of light for 14 days and for analysis, samples were taken at Day 0 and 14.

## Results and Discussion

**Table 1.** Evaluation of fiber substances content of the examined substrates

| Ratios OMSW - WS | Crude Fiber Substances (%) | | Cellulose (%) | | Lignin (%) | |
|---|---|---|---|---|---|---|
| (%w/w) | *Day 0* | *Day 14* | *Day 0* | *Day 14* | *Day 0* | *Day 14* |
| 100 OMSW | 39.31±0.22 | 43.65±0.33 | 18.71±0.43[e] | 32.78±0.06[f] | 35.26±1.45[k] | 27.08±1.37[l] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 80-20 | 49.67±1.08 | 45.50±0.32 | 27.06±1.39[g] | 34.32±0.37[h] | 33.27±1.09[m] | 26.16±0.50[n] |
| 60-40 | 48.09±0.89 | 51.79±1.09 | 30.95±1.31 | 33.18±1.04 | 30.61±1.68 | 29.41±1.03 |
| 40-60 | 53.94±1.26 | 56.00±0.20 | 27.76±1.87[i] | 35.12±1.42[j] | 36.47±1.44[o] | 26.94±1.46[p] |
| 20-80 | 64.04±1.20[a] | 48.88±1.42[b] | 35.16±1.34 | 32.81±1.28 | 33.47±1.01[q] | 27.84±0.65[r] |
| 100 WS | 70.20±0.02[c] | 50.71±0.45[d] | 40.42±0.80 | 36.76±1.10 | 31.40±1.25 | 26.79±1.63 |

Statistical analysis was performed for each examined parameter and substrate individually between Day 0 and Day 14. Different superscripts indicate statistical significance ($p \leq 0.05$)

Table 1, presents the fiber substances' profile of the examined substrates, which were analyzed during the mycelium growth, from the beginning (Day 0) until the end (Day 14) of the fermentation process. In specific, crude fiber substances' content, varied between the examined ratios by WS's addition of 60%w/w recording the highest value, whereas the substrate of 100%w/w of WS revealed a statistically significant reduction ($p \leq 0.05$) by 27.76% at the end of the process. Cellulose concentration followed a similar pattern with the proportion of 100% w/w of OMSW presenting the highest statistically significant increment ($p \leq 0.05$) by 75.2%, while the substrate of 100%w/w of WS revealed the highest reduction by 9.05%. Lignin profile showed a different behavior, since latter's content was found to be decreased to all the examined ratios by WS's addition of 80%w/w to OMSW displaying the highest statistically significant reduction ($p \leq 0.05$) by 26.13% between Days 0 and 14.

**Table 2.** Assessment of proteins and β-glucans content

| **Ratios** | **Proteins (%)** | | **1,3-1,6 β-Glucans w/w (%)** | |
|---|---|---|---|---|
| OMSW – WS (% w/w) | *Day 0* | *Day 14* | *Day 0* | *Day 14* |
| 100 OMSW | 7.07±0.50 | 7.58±0.31 | 2.83±0.14[e] | 4.86±0.32[f] |
| 80-20 | 6.54±0.03 | 7.57±0.12 | 2.40±0.23 | 2.73±0.11 |
| 60-40 | 6.01±0.16 | 6.49±0.63 | 1.79±0.21[g] | 4.02±0.54[h] |
| 40-60 | 4.94±0.08 | 5.16±0.29 | 2.09±0.08[i] | 6.94±0.61[j] |
| 20-80 | 4.47±0.12[a] | 7.37±0.51[b] | 2.25±0.12[k] | 5.46±0.19[l] |
| 100 WS | 2.63±0.18[c] | 5.97±0.13[d] | 3.62±0.11[m] | 4.95±0.47[n] |

Statistical analysis was performed for each examined parameter and substrate individually between Day 0 and Day 14. Different superscripts between indicate statistical significance ($p \leq 0.05$)

According to Table 2, proteins and β-glucans content was found to be increased to all the examined substrates at the end of the fermentation process. More specifically, in the examined substrate of 100%w/w of WS proteins presence exceeded a 2-fold statistically significant increase ($p \leq 0.05$), whereas concerning β-glucans concentration WS's addition by 60%w/w to OMSW, displayed a statistically significant increment ($p \leq 0.05$) ranging from 2.09% (Day 0) to 6.94% (Day 14).

## Conclusions

Study herein highlighted the importance of novel raw materials' utilization (OMSW, WS and their mixtures) for the production of innovative proteinaceous animal feed. Results herein are indicative of the aforementioned substrates' capability to be bioconverted into crude protein enriched products with enhanced β-glucans content, upgrading thus their nutritional composition in order to be served as suitable supplements for feedstuffs. The presented herein exploitation of agro-industrial wastes demonstrates a pathway that simultaneously confronts various environmental issues and contributes in the context of the circular economy.

## References

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