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

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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*.

Results & Discussion

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 1. Evaluation of fiber substances content of the examined substrate

Ratios OMSW - WS (%w/w)	Crude Fiber Substances (%)		Cellulose (%)		Lignin (%)	
	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 between indicate statistical significance ($p \leq 0.05$)

Considering our results, WS additions to OMSW recorded a reduction concerning lignin content due to the efficiency of *P. ostreatus* to act as lignin degradation agent by secreting oxidative enzymes, such as Laccases, Lignin peroxidases and Manganese peroxidases. This fact secures the ability to improve the substrates' digestibility, making them suitable for feedstuffs.

Table 2 summarizes proteins and β-glucans content of the examined substrates

Table 2. Assessment of proteins and β-glucans content

Ratios OMSW – WS (% w/w)	Proteins (%)		1,3-1,6 β-Glucans 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). Proteins increment could be associated with the potential uptake of nitrogen excess which is binded and bio-converted by the microorganism into protein content through aerobic fermentation. Another possible explanation of increased protein content can be related to the secretion of particular proteinaceous extracellular enzymes which contribute to the materials' degradation during the fermentation procedure. Finally, the increased β-glucans content to the fermented substrates is associated with *P. ostreatus* biochemical process who is utilizing substrates' nutrients, resulting thus to their increment.

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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