Research Article

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Effect of different lignocellulosic wastes on *Hericium americanum* yield and nutritional characteristics

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Abstract

BACKGROUND: The aim of this study was to investigate the possibility of using cottonseed hulls (CSH) and olive press cake (OPC) as new supplement materials for substrate preparation in *Hericium americanum* cultivation. Some chemical properties of the substrates prepared by mixtures of oak sawdust (OS) with wheat bran (WB), CSH and OPC in different ratios were determined. In addition, the effect of mixtures of OS:CSH and OS:OPC on spawn run time, yield and biological efficiency (BE), average mushroom weight and nutrition content of the fruiting body were compared with the control substrate (8OS:2WB).

RESULTS: The yield, BE and average mushroom weight of substrates containing CSH and OPC were higher than the control substrate and increased with an increase in the rate of CSH and OPC in the mixtures. *Hericium americanum* showed (on a dry weight basis) 8.5–23.7% protein, 9.9–21.2 g kg^{-1} P, 26.6–35.8 g kg^{-1} K, 0.63 – 1.33 g kg^{-1} Mg, 0.19 – 0.23 g kg^{-1} Ca, 1.34–1.78 g kg^{-1} Na, 49.5–72.2 mg kg^{-1} Fe, 6.22–10.11 mg kg^{-1} Mn, 32.8–82.8 mg kg^{-1} Zn and 8.6–11.2 mg kg^{-1} Cu on different growing substrates. The nutritional value of mushrooms was greatly affected by the growing media.

CONCLUSION: The results revealed that CSH and OPC could be used as new supplement materials for substrate preparation in *H. americanum* cultivation.

Keywords: *Hericium americanum*; wheat bran; cottonseed hulls; olive press cake; macro- and micro-element content

INTRODUCTION

Lignocellulosic residues comprise a broad range of by-products from agricultural and forest industries. The disposal of these wastes is crucial because of their chemical structure and decomposition properties. Cottonseed hulls and olive press cake are by-products of cotton and olive production in the Mediterranean Basin. Their use as substrate for mushroom cultivation could present extensive opportunities in processing procedures for waste utilization and the production of value-added products. Previous research has shown that the cottonseed hull possesses advantages as a substrate material because of its higher water retention capability and nitrogen content,[1] leading to higher mushroom yield.[2–4] Moreover, some species of mushroom were able to colonize used growing media, supplemented with olive by-products, and produce fruiting bodies.[5,6]

Hericium spp. are white-rot fungi belonging to the family Hericiaceae. *Hericium* spp. are efficient lignin-degrading mushrooms that are able to colonize different types of agricultural wastes as substrates. The effects of different basal and supplement materials on the yield of *Hericium* spp. have been investigated in different studies. Thus Eisenhut and Fritz[7] suggested that sawdust was an efficient basal substrate. Hassan[8] researched the incubation time and yield of *Hericium erinaceus* on six different substrate formulations containing sawdust, rice straw and wheat straw, in various combinations with wheat bran, finding the sawdust and wheat bran combination was the best. Akdeniz[9] investigated the suitability of wheat bran, tea waste, sunflower bagasse, rice bran and chicken manure as supplements for *H. erinaceus* cultivation. Furthermore, some data and reference texts concerning the cultivation of *H. erinaceus* on cottonseed substrate are available.[10]

Limited data and reference texts are also available on the physiological, genetic and cultural characteristics of *H. americanum*, and there is only one report on the evaluation of the use of agricultural by-products with *H. americanum*. Rice bran, wheat bran, barley bran, Chinese cabbage, eggshell and soybean powder were investigated as supplement materials for *Hericium* species, including *H. americanum*, by Ko *et al.*[11] Soybean powder was found to be the best supplement for *H. americanum* growth in that study.

On the other hand, the composition of mushrooms also depends on the growing medium and cultivation conditions.[12] According

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to Ragunathan *et al.*,[13] knowledge of the relationship between the properties of the mushrooms and agricultural waste characteristics may contribute to the improvement in quality of the products. More information about *H. americanum* is necessary to screen efficient substrates and to improve mushroom yield and quality. Based on that, the objectives of this work were: (i) to compare yield and the nutrient composition of *H. americanum* grown on different substrates; (ii) to discover the relationship between this composition of mushroom and its nutrient source; and (iii) to investigate the suitable substrate for the production of high-quality mushrooms.

MATERIALS AND METHODS

Materials

Agricultural wastes were obtained from local markets (Menemen, Izmir, Turkey). Pure cultures of *H. americanum* were provided from the collections of the Agroma Co. Ltd, Turkey. The cultures were maintained in a malt extract agar (MEA) medium and stored in a refrigerator at 4 °C. This study was conducted at the Mushroom Production Unit of Ege University's Faculty of Agriculture in Izmir, Turkey. Analysis was carried out in the Research Unit Sustainable Use, Management and Reclamation of Soil and Water (GARSA) Laboratories at the Technical University of Cartagena (Spain).

Experimental design

Seven different growing media were tested for the cultivation of *H. americanum*. Oak sawdust (OS) was used as a base medium, and cottonseed hulls (CSH) and olive press cake (OPC) were added to sawdust to prepare the growing media. Three combinations of OS + CSH and three of OS + OPM at ratios of 9:1, 8:2 and 7:3 were used. Sawdust substrate, supplemented with wheat bran in a ratio of 8:2 (commercial formulation for *H. erinaceus*), was used as a control medium. The experiment was conducted in a randomized plot design, with ten replications. The agro-residues were analysed in the aforementioned GARSA laboratories.

Preparation of cultivation media

Each substrate was thoroughly mixed, and distilled water was added until it was moistened to 70%. Then, 1 kg (wet weight) of each substrate was packed into a polypropylene autoclavable bag of 25 × 45 cm and the bag plugged with a cotton plug. Ten replicates were performed for each growing medium formulation. The plastic bags containing substrate were sterilized in an autoclave at 121 °C for 90 min and, after cooling, inoculated in a laminar flow chamber using 3% grain spawn (on a w/w wet weight basis).

Mushroom cultivation

Inoculated bags were incubated at 25 ± 2 °C with 80% relative humidity in the presence of light to be colonized by the mycelium. After full colonization, bags were transferred to a cropping room at 20 ± 2 °C with a humidity of 80–90% in order to induce fructification. The cotton plugs were removed and the tops of the bags were folded down. Cool white fluorescent bulbs provided 8 h of light daily. Sufficient air changes were maintained to hold CO_2 concentration below 500 mg kg^{-1}. Total mushroom yield (g kg^{-1} substrate) was obtained from two flushes in a harvest period of 60 days. Mushrooms were harvested from the bags when the branches had fully expanded and individual spines were partially formed (around 5 mm long).

The biological efficiency percentage (BE) was calculated as follows: ([weight of fresh mushrooms harvested/substrate dry matter content] × 100).[14]

Macro- and micro-element analysis

Mushroom samples were oven-dried at 60 °C for 48 h and ground to pass through a 1 mm sieve. In order to determine nutrient and phosphorus content in samples, 0.7 g mushroom samples were weighed and incinerated in a muffle furnace at 550 °C for 16 h; then ash residue was digested in 0.6 mol L^{-1} nitric acid (HNO_3). Finally, the solution was used for the determination of nutrients by an atomic absorption spectrometer (AAS) (AAnalyst 800 AAS, PerkinElmer Inc., Waltham, MA, USA), and phosphorus by a spectrophotometer.

The Duchaufour[15] method was used to determine the total nitrogen content of each mushroom. The Ca and Mg content was each measured by flame atomic absorption; while Na and K were measured by emission with an AAS (AAnalyst 800); and phosphorus (PO_4^{-3}) was measured in accordance with the MAPYA[16] method. The crude protein content was calculated by using the adjusted conversion factor for mushroom (N × 4.38).[17] The Cu, Zn, Fe and Mn content was each measured by an AAS (AAnalyst 800) according to the method used by Madrid *et al.*[18]

Statistical analysis

The data obtained from the experiment were subjected to variance and means analysis, and the statistical significance was compared employing Duncan's multiple range test, using the SPSS 16.0 for Windows statistical computer program at a significance level of 5%.

RESULTS AND DISCUSSION

Growing media

Significant differences were found among the growing media regarding ash, C and N content and C:N ratio. The ash content of 7OS:3CSH (8.63%) was the highest, while the N content of substrates varied between 0.26% (9OS:1OPC) and 0.88% (7OS:3CSH) (Table 1). The C:N ratio of growing media varied between 51.7% and 178.81% and it was noted that the C:N ratio decreased when the amount of CSH and OPC in the substrate increased. This could be explained by the increased N content in the growing medium of these substrates. Kalberer[19] reported that the C:N ratio depends on the availability and concentration of both the C and N sources.

Significant differences were found among the P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu content of the growing media ($P < 0.01$). Jonathan and Fasidi[20] reported that minerals such as Ca, Mg and K, Cu and Zn are required by fungi for high mycelial yields and fruiting body production. Levels of P, Ca, Mg, Na, Fe, Mn and Cu content of the growing media supplemented with CSH were higher than that of other media, while the level of K and Zn content in the control substrate was the highest. Macro- and micro-element content increased when the rate of CSH was increased from 10% to 30%. Among the growing media, that supplemented with OPC had the lowest macro- and micro-element content. The level of Cu content was highest in OS:OPC substrates. This can be attributed to the low mineral content of the OPC used for substrate preparation initially (data not shown).

Table 1. Chemical composition of different growing media used in the study

Properties	Substrate						
	8OS:2WB	9OS:1CSH	8OS:2CSH	7OS:3CSH	9OS:1OPC	8OS:2OPC	7OS:3OPC
Ash (%)	6.4bc**	5.4c	6.17bc	8.6a	5.8bc	5.3c	6.5b
C (%)	46.8bc**	47.3ab	46.9abc	45.68d	47.09abc	47.33a	46.77c
N (%)	0.68b**	0.57c	0.60c	0.88a	0.26e	0.32d	0.33d
C:N	68.8c**	82.7c	78.3c	51.7d	178.8a	147.1b	146.8b
P (mg kg^{-1})	0.21d**	0.29b	0.30c	0.40a	0.11e	0.13e	0.14e
K (mg kg^{-1})	0.32a**	0.28b	0.27b	0.27b	0.17d	0.22c	0.26b
Ca (mg kg^{-1})	2.56c**	3.13 b	3.35 b	3.88 a	1.93d	1.88d	1.85d
Mg (mg kg^{-1})	0.108b**	0.087c	0.096bc	0.127a	0.039d	0.038d	0.037d
Na (mg kg^{-1})	0.019c**	0.018c	0.026b	0.036a	0.011d	0.011d	0.013d
Fe (mg kg^{-1})	119.4e**	288.1b	284.9b	330.4a	133.7e	151.4d	178.8c
Mn (mg kg^{-1})	53.5c**	53.5c	56.5b	63.1a	48.3d	43.9e	35.4f
Zn (mg kg^{-1})	78.8a**	61.2b	58.0c	61.1b	54.8d	51.6e	57.2 cd
Cu (mg kg^{-1})	8.9c**	8.7c	9.5c	11.8a	10.5b	11.5a	12.2a

Asterisks indicate significance at $^{*}P < 0.0.05$, $^{**}P < 0.0.01$; values within the same row followed by the same letter are not significantly different. Mean values in the same row followed by the same letters are not significantly different by Duncan's multiple range test.

Table 2. Effect of different growing media on spawn running time, yield, BE and average mushroom weight of *Hericium americanum*

Treatment	Spawn running time (days)	Yield (g kg^{-1} substrate)	Biological efficiency (%)	Average mushroom weight (g)
8OS:2WB	28.9c**	119.02d**	34.55d**	59.51d**
9OS:1CSH	28.5c	149.53c	43.40c	74.77c
8OS:2CSH	26.6e	215.92a	62.67a	93.17a
7OS:3CSH	27.1de	200.32ab	58.14ab	100.16a
9OS:1OPC	28.2 cd	155.65c	45.18c	77.83bc
8OS:2OPC	30.1b	179.79b	52.19b	89.90ab
7OS:3OPC	33.3a	190.78b	55.38b	95.39a

Asterisks indicate significance at $^{*}P < 0.0.05$, $^{**}P < 0.0.01$; values within the same row followed by the same letter are not significantly different. Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.

Yield

Spawn run time, yield, BE and average mushroom weight were all affected by the growing media ($P < 0.01$).

Spawn running time varied between 26.6 and 33.3 days, depending on the growing media (Table 2). Spawn run time is influenced by other factors such as the strain, substrate formula, amount of substrate available, spawning rate, spawn distribution and temperature during incubation.[21,22] Oei[23] reported that the spawn run time of *H. erinaceus* was 6 weeks at 20 °C, while Hassan[8] confirmed that this varied, in different growing media, between 37 and 46 days. Compared to previous findings, the spawn run time of *H. americanum* is shorter than that of *H. erinaceus*. Ko *et al.*[11] also reported that mycelial growth of *H. americanum* was faster than other *Hericium* spp.

In general, the C:N ratio plays a crucial role in the mycelial growth rate and also in the formation of fruiting bodies.[24] The C:N ratio was suggested at 50:1 for different substrates by Balakrishnan and Nair.[25] It was found that the medium 8OS:2CSH represented the best growing medium for mycelial growth of *H. americanum*, followed by 7OS:3CSH. The formulated substrates with C:N ratios ranging between 51.7 and 82.7 showed increased mycelial growth. However, Naraian *et al.*[26] reported that combinations having higher concentrations of supplement materials did not improve the growth rate. The spawn running period for mixtures of OPC with OS was longer than that of other mixtures.

Total yield of *H. americanum* in the seven substrates varied between 119.02 and 215.92 g kg^{-1} substrate, while BEs varied between 34.6% and 62.67%. Mature fruiting bodies grown on all growing media are shown in Fig. 1. Ko *et al.*[11] reported that the yield and BE of *H. americanum* were 243 g per 1100 g bottle, and 43%, respectively – close to our values. In the present study, the yield and BE in the growing media containing OPC increased with increasing OPC content in the substrates. A minimum average yield and BE were obtained in the control medium. The 8OS:2CSH combination gave the higher yield, which was 81.4% higher than control. Similar results have been reported by He *et al.*[27] when 20% (w/w) of cottonseed hull was added to a rice straw substrate, resulting in a 20% increase in mushroom yield of *Pleurotus ostreatus*, while Wang[28] reported that 28–56% (w/w) of cottonseed hull added to a wheat substrate resulted in a 15–18% increase in mushroom yield of *Agaricus blazei*. These results are consistent with our findings, where CSH supplementation to sawdust significantly improved the yield. Moreover, the advantages of supplementing OPC in the substrate mixture have already been reported elsewhere for the cultivation of some mushroom species such as shiitake[29] and *Pleurotus* spp.[4,22,30]

The 7OS:3CSH substrate contained 0.88% N at its highest value, and the highest mushroom yield has been obtained with this medium together with 8OS:2CSH. However, the 9OS:1OPC substrate, with the lowest N (0.26%), gave the lowest yield, followed

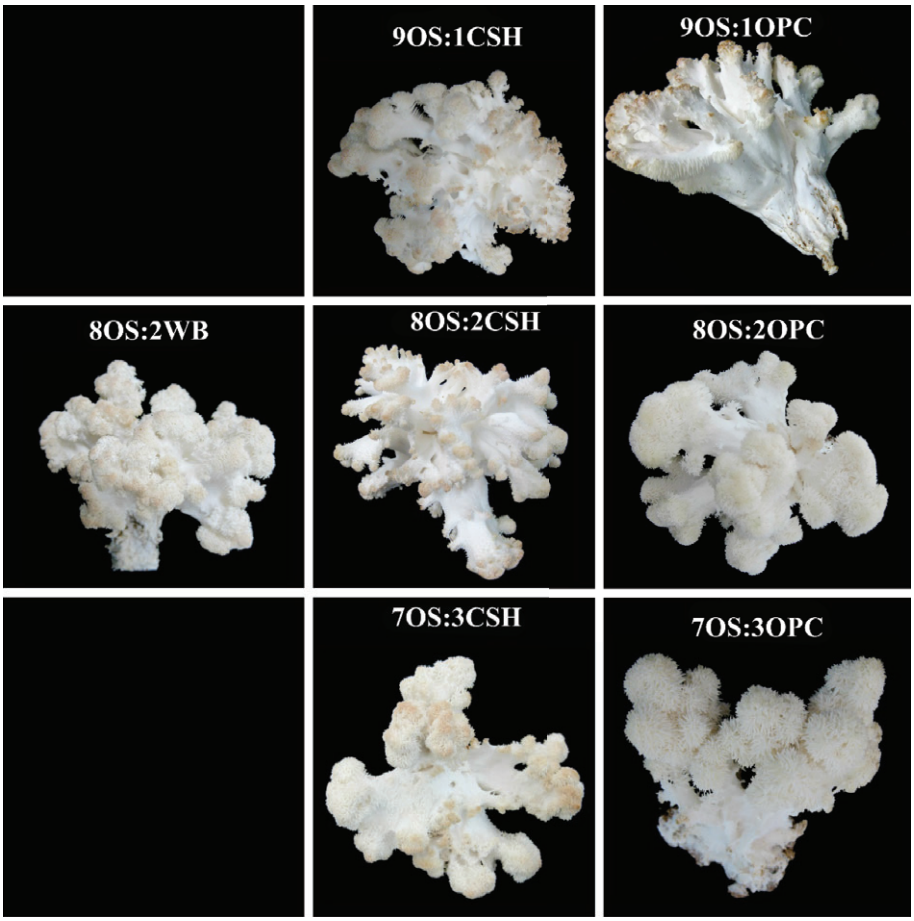

Figure 1. Fruiting bodies of *Hericium americanum* grown on different growing media.

by control medium. On the other hand, some rich nitrogen sources may not produce yields as expected, such as happened with wheat bran. The second highest N level (0.68%) was in the 8OS:2WB combination, and this growing medium gave the lowest yield. Moreover, Olivier[31] demonstrated that the highest yield can be obtained from the substrate that contains 0.7–0.9% N in dried weight or with a C:N ratio of 50:1 or higher. Our findings were confirmed by those of Olivier[30] and Balakrishnan and Nair.[25] The highest average mushroom weight values were recorded on substrates of 7OS:3CSH, 7OS:3OPC and 8OS:2CSH, followed by 8OS:2OPC. There was a positive relation between average mushroom weight and high mushroom yield (data not shown). Our results are corroborated by the findings of Philippoussis *et al.*,[32] who reported that the highest BE and the heaviest fruiting bodies were obtained on the same growing medium.

On the other hand, the mycelial growth rate of *H. americanum* was not correlated with the production of mushrooms. In the study carried out by Zervakis *et al.*,[21] the linear growth rates of seven species of mushrooms were compared with the yield produced on the substrates that showed the fastest mycelial growth. Only three of the seven isolates had the highest yield on the substrate that showed the fastest mycelial growth. The authors reported that fast mycelial extension was often interpreted as an indication of hyphal progression on a nutritionally poor or unfavourable medium. A slower and denser growth can be attributed to favourable conditions and the exploitation of the nutrient resources of the medium by the fungus. This could lead us to hypothesize that fast growth rate is actually inversely related to yield.[22] Moreover, Philippoussis *et al.*[32] found a negative correlation between growth rate and biomass yield of *Lentinula edodes*. This could lead us to hypothesize that fast growth rate is actually inversely related to yield.

Protein, macro- and micro-element content of fruiting bodies

The protein, P, K, Mg, Ca, Na, Fe, Mn and Zn content of fruiting bodies was affected by the different substrates ($P < 0.01$), although the ash content was unaffected ($P > 0.05$). The ash content of fruiting bodies was found to be between 7.69% and 8.16%, based on dry weight. The maximum ash content of *H. americanum* was found in the 8OS:2CSH combination, followed by the 7OS:3CSH combination. The values found were lower than the ash content presented by Hassan,[8] with 9.69–11.27% for *H. erinaceus*, although similar to that shown by Akdeniz[9] in the same species (7–9%). The protein content of fruiting bodies was between 6.0% and 11.6%, depending on growing media (Table 3). The supplemented sawdust with wheat bran had a greater potential to improve the accumulation of protein than supplementation with CSH and OPC. Our findings are close to the protein content of *H. erinaceus*[8,9,33,34] in various growing media. According to Wang *et al.*,[35] the supplementation with wheat bran had a greater potential to improve the accumulation of protein in *Pleurotus ostreatus* than did rice bran or corn bran. Khan *et al.*[36] determined that in most cases the high protein content of mushrooms is correlated with the high protein content in substrates. In our study, the 7OS:3CSH growing medium presented

Table 3. Effects of different growing media on nutrient content of fruiting body of *Hericium americanum*

	Ash (%)	Protein (%)	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca (g kg^{-1})	Na (g kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
8OS:2WB	7.98 ns	11.6a**	21.2a**	33.8b**	1.33a**	0.23a**	1.60b**	72.2a**	9.05b**	82.8a**	11.2a**
9OS:1CSH	8.01	7.6d	15.4d	29.9c	0.93d	0.21bc	1.78a	32.1e	10.11a	57.0b	10.0c
8OS:2CSH	8.16	10.0c	17.8c	34.2ab	1.08c	0.20c	1.46c	49.5d	7.80c	56.5b	11.1a
7OS:3CSH	7.95	11.5b	18.9b	35.8a	1.16b	0.22ab	1.34d	65.1b	8.60b	57.0b	10.5b
9OS:1OPC	7.69	6.0f	9.9 g	26.6e	0.82e	0.20b	1.35d	51.1d	8.46b	59.1b	8.6e
8OS:2OPC	7.69	6.7e	11.5f	28.7 cd	0.63f	0.21b	1.33d	53.6d	6.22e	32.8d	9.00d
7OS:3OPC	7.90	8.1d	12.3e	28.0de	0.68f	0.19c	1.35d	58.6c	7.06d	46.5c	8.97de

ns, not significant; Asterisks indicate significance at $^{*}P < 0.05$, $^{**}P < 0.0.01$; values within the same row followed by the same letter are not significantly different. Mean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test.

the largest percentage of nitrogen; however, the result demonstrated that fruiting bodies cultivated on 8OS:2WB had the highest protein content. Our findings are confirmed by Wang *et al.*,[35] who reported that not only the amount but also the nature of the N source present in the substrates influences the protein content of fruiting bodies. Also, the protein content increased with an increase in the rate of CSH and OPC. The figures for protein content were 7.6% and 6% when the amount of CSH and OPC was 10%, with the highest values of 11.5 % and 8.1% observed at around 30% CSH and OPC, respectively.

K and P were the main constituents of ash. K was the most abundant mineral element in the cultivated mushroom samples (26.6–35.81 g kg^{-1} dry weight (dw)), 7OS:3CSH being the best growing medium. Gençcelep *et al.*[37] reported the K content of wild edible mushrooms as being between 12.6 and 29.1 g kg^{-1} dw, although Sanmeea *et al.*[38] found that this element could rise to 45.2 g kg^{-1} dw. *P*-values recorded in mushrooms were between 8.5 and 23.74 g kg^{-1} dw. This result is compatible with those of previous reports.[39,40] The highest P content was obtained on control growing media. Mg represented the third major element, ranging from 0.64 to 1.34 g kg^{-1} dw. The Mg content obtained was generally in accordance with those given in previous publications.[34] Concentrations of Ca ranged from 0.19 to 0.23 g kg^{-1} dw, similar to those obtained by Sanmeea *et al.*[38] (0.100–2.4 g kg^{-1} dw). Fruiting bodies grown on 8OS:2WB had more Ca content than those grown on other combinations.

Fe and Zn have been reported to be the most abundant micro-elements encountered in various species of edible mushrooms. These elements are important for the normal growth and development of humans, and mushrooms are known as important accumulating species of these elements.[41] In the literature, Fe values in the dry weight of mushrooms have been recorded as 31.3–1190 mg kg^{-1},[42] and 56.1–7162 mg kg^{-1}.[43] However, as in vegetables, Fe was present in low concentrations (49.5–72.23 mg kg^{-1}). The Fe content of fruiting bodies cultivated in 8OS:2WB substrate was the highest, while the Zn content of fruiting bodies cultivated in control substrate (82.84 mg kg^{-1}) was the highest. The results obtained for Zn content are in accordance with the literature, which has reported such content in the range of 22.1–214.3 mg kg^{-1} dw.[37,44–46] The Mn content ranged from 6.23 mg kg^{-1} dw (8OS:OPC) to 10.12 mg kg^{-1} dw (9OS:1OPC), while the Cu content of the samples varied from 8.59 mg kg^{-1} (9OS:1OPC) to 11.17 mg kg^{-1} dw (8OS:2WB). The Mn and Cu values in this study are in agreement with results found in the literature.[37,47] Cu accumulation was highest on the control media, while the Mn accumulation was highest on 9OS:1CSH. The fruiting bodies grown in OS:OPC media exhibited the lowest mineral content for most macro- and micro-elements, which can be explained by their low content in the OS:OPC substrate.

Protein, P, K, Ca and Mg were the major elements found in the cultivation substrates and in fruiting bodies (Tables 1 and 3). Concentrations of these elements were higher in fruiting bodies than in substrates. The intake of elements by mushrooms differed among minerals. It was observed that mushrooms accumulated N, P, K, Mg, Na and Cu, while they filtered Ca, Fe and Mn. Fruiting bodies were especially effective in N and K accumulation, despite a very low content of these elements in the substrate. K in the cultivation substrate was transferred very efficiently to the fruiting body. K levels were between 5.3 and 14.5 times higher in fruiting bodies than in the substrates; this is lower than in previously reported studies (20–105).[41,48] Ca and Mg levels in fruiting bodies were lower than in the substrates. Kalac[48] and Jo *et al.*[49] reported similar results in *Ganoderma lucidum* and some wild mushrooms. Cu was accumulated in fruiting bodies. On the other hand, levels of Zn were similar in the fruiting body and in the substrate. This result also corroborates the earlier findings of Kalac and Svoboda.[44]

Some previous studies[24,50] reported a direct effect of the chemical composition of the growth medium on the chemical composition of the fruiting body, but most data from our study did not indicate whether the composition of the substrate influences the mineral content of the fruit body. Our findings support the conclusion of Silva *et al.*[51] in that the substrates used for cultivation influenced the chemical composition of the mushrooms. However, their chemical composition did not correspond in all the cases to the chemical composition of the substrate, although in many cases it was well correlated.

CONCLUSION

These study results demonstrated that olive press cake and cottonseed hulls can be used as a new and economic supplement material in the artificial cultivation of *H. americanum*. The differences in levels of protein and mineral elements can be explained by the nutritional value of mushrooms, which can be greatly affected by the cultivation substrates. It is evident that the supplementation of OPC was effective in improving yield, BE and average mushroom weight. Moreover, OPC can be considered ecologically practical and economically feasible owing to its availability in large quantities throughout the year at little or no cost, in the Mediterranean Basin. On the other hand, fruiting bodies grown on OS:OPC medium exhibited low protein and mineral content. Therefore, different combinations should be investigated in more

detail to improve the nutritional content of fruiting bodies grown on OS:OPC combinations. WB seems to be the best supplement among other agricultural residues for improving the nutritional content of *H. americanum* fruiting bodies. It is clear that WB was effective in increasing the protein content in fruiting bodies. A mixture of OPC and WB can be researched as supplement material to improve the crude protein content of fruiting bodies. Furthermore, different materials and mixing ratios should be investigated in more detail to increase mushroom yield and improve the nutritional content of *H. americanum*.

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