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Using Phenol-Rich Agro-Wastes as Substrates for the Cultivation of *Hypsizygus ulmarius* Mushroom with Enhanced Functional and Nutritional Potential

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HIGHLIGHTS

- Straw-based media are more suitable for *Hypsizygus ulmarius* cultivation
- Grape pomace and green walnut husks are useable as alternative supplement materials
- These harmful wastes are converted into functional foods by mushroom cultivation

Abstract: This study aimed to transform phenolic-rich agricultural wastes into valuable foods by using them as new alternative substrates in mushroom cultivation and thus, to dispose of them without harming the environment. Thirteen growing media were tested for cultivation of *Hypsizygus ulmarius* in the study. The wheat straw (WS) and poplar sawdust (PS) were used as the main substrate and the green walnut husk (GWH) and grape pomace (GP) were added at the ratios of 10, 20, and 30%. One commercial growing medium was used as control. The relationships was assessed between the cultivation parameters and composition of the growing media. Moreover, the comparison of nutritional composition of mushroom carpophores among the treatments was performed. Straw-based media supplemented with GP and GWH promoted *H. ulmarius* yields (161.1 g kg^{-1}–235.4 g kg^{-1}), biological efficiency (BE)% (44.8%–73.5%) compared to control media (225.7 g kg^{-1}, 64.5%, and 1.25, respectively). Moreover, the fruitbodies cultivated on growing media supplemented with different ratios of GP and GWH displayed high contents of protein (12.85%–17.0%), ash (8.54–11.90%), and carbohydrate (69.11%–75.15%), a total phenolic content of 2.68–4.26 mg gallic acid equivalents (GAE) per g, and a low content of fat (1.70%–3.50%). Considering the results of the study, it was concluded that by using growing media containing phenolic-rich wastes in *H. ulmarius* cultivation, especially GP, the environmental impact of these wastes can be reduced without compromising the current yield performance and nutritional quality of the mushroom.

Keywords: Elm oyster mushroom; grape pomace; green walnut husk; total phenol; agro-wastes.

INTRODUCTION

Walnuts and grapes, which have been used by humans for thousands of years, are also crops of major economic importance in the present day. The walnut kernel industry generates large quantities of shells and green husks that are managed as waste. Yilmaz and coauthors [1] reported that different walnut cultivars produced between 170 and 290 g of dry green husk biomass for every unit of dry inshell biomass. Considering

that the worldwide walnut production in 2019 was approximately 4.498.442 tons [2], the biomass of green walnut husk (GWH) that emerged in the same year was calculated as approximately 383.000–520,000 metric tons. Grape pomace (GP) is an important by-product of wine, molasses, and vinegar production and contains 17 polyphenols such as gallic acid, catechin, epicatechin etc. [3]. As a result of these production processes, the grape pulp generated is approximately 25% of the weight of the grapes processed, and the total amount of this waste is over 9 million tons annually [4].

As they have little or no use, GWH and GP represent wastes that have a great negative impact on the environment. The accumulated GWH and GP not only threaten the environment, but also require considerable labor and space for their disposal. Waste management is one of the most important tools for preventing environmental pollution. Therefore, in terms of ecological and economic benefits, the disposal of wastes with value-added recycling technologies is encouraged. Mushroom cultivation that combines the production of therapeutic and nutritious food along with the disposal of agricultural wastes rich in phenolic content could be a safe and reliable way for the elimination of the potentially toxic wastes resulting from the production of the walnut and grape industries.

The use of phenolic-rich agricultural wastes for the cultivation of different mushroom species has been examined in a number of previous studies. These have utilized the wastes generated by the olive oil, wine, and coffee industries [5,6,7,8,9] and have provided promising results, not only for the production of mushrooms, but also for the disposal of agricultural wastes rich in phenolic content. Phenolic compounds exhibit strong antioxidant, antitumoral, and antimicrobial activity [10]. This highlights the importance of conducting studies to raise the content of these compounds in the mushroom carpophore. Some data are found in the literature concerning the effect of the chemical content of substrates on the nutritional value and antioxidant activity of various mushroom species [11]. Therefore, natural sources of antioxidants can be produced via the selection of substrates with high phenolic content for mushroom cultivation.

Hypsizygus ulmarius is a mushroom that is widely cultivated worldwide, especially in Asia and Europe, because of its high biological efficiency and easy and cheap production technology [12]. *H. ulmarius* is commonly cultivated on straw-based substrates, e.g., paddy straw and wheat straw. Of late, some non-traditional basal substrates such as banana leaves, water hyacinth, groundnut shells, sawdust, and sugarcane waste [13], with supplemental materials utilized as substrate for *H. ulmarius* such as banana leaves [14], biogas digester liquid [15], and seaweed [16]. However, to the best of our knowledge, no study has been conducted on the utilization of GP and GWH for the cultivation of *H. ulmarius*. For that reason, the current study evaluated the possibility of using polyphenol-rich agro-food wastes as supplementation materials to improve the yield and productivity as well as the nutritional and phenolic content of *H. ulmarius*.

The main purpose of the research was to investigate the recycling of biotoxic GWH and GP for nutrimental food production through mushroom cultivation technology. Our specific objectives were (1) to compare three different ratios of GP and GWH and (2) to determine the optimum GP and GWH concentrations that would provide increased mushroom productivity and enhance the nutritional and functional properties of *H. ulmarius.*

MATERIAL AND METHODS

Strain and spawn

The strain of *H. ulmarius* was supplied by Homegreen Spawn Company, The Netherlands. It was temporarily maintained on malt extract agar (MAE) and stored at 4ºC until use. The mycelium was inoculated into wheat grains to be used as spawn and prepared as previously described by Atila [17].

Substrates

The agricultural waste wheat straw (WS), GWH, and wheat bran (WB) used were gathered from local farms in Kırşehir, Turkey, whereas the GP was obtained from a local winery. Poplar sawdust (PS) was purchased from a lumber mill in Kırşehir.

Preparation of cultivation substrates

The preparation of substrates was based on the dry weight of each component before mixing. The GP and GWH were dried and crumbled into small pieces (approx. 0.1-0.2 cm). The WS was chopped into small pieces (2–3 cm).

Basal and supplemental materials were prepared in thirteen different ratios and tested as *H. ulmarius* growing media (Table 1). To prepare the growing media, the WS and PS were used as the main substrate

and the GWH and GP were added at the ratios of 10, 20, and 30%. A commercial growing medium containing 80% WS and 20% WB was used as the control substrate.

Table 1. Formulations of growing media and ratios of basal substrate and supplement material used in the study.

Growing Media	Basal Substrate	Basal Substrate Ratio (w/w)	Supplement material	Supplement Material Ratio (w/w)
Control	Wheat straw	80%	Wheat bran	20%
WS:GWH10	Wheat straw	90%	Green walnut husk	10%
WS:GWH20	Wheat straw	80%	Green walnut husk	20%
WS:GWH30	Wheat straw	70%	Green walnut husk	30%
WS:GP10	Wheat straw	90%	Grape pomace	10%
WS:GP20	Wheat straw	80%	Grape pomace	20%
WS:GP30	Wheat straw	70%	Grape pomace	30%
PS:GWH10	Poplar Sawdust	90%	Green walnut husk	10%
PS:GWH20	Poplar Sawdust	80%	Green walnut husk	20%
PS:GWH30	Poplar Sawdust	70%	Green walnut husk	30%
PS:GP10	Poplar Sawdust	90%	Grape pomace	10%
PS:GP20	Poplar Sawdust	80%	Grape pomace	20%
PS:GP30	Poplar Sawdust	70%	Grape pomace	30%

The substrates were mixed and their moisture contents were adjusted to about 65-70% by adding tap water. The prepared growing media in amounts of 1 kg were then placed in polypropylene bags (25×45 cm) and autoclaved at 121°C for 90 min. After cooling, each bag was inoculated with 3% (w/w) mushroom spawn on the wet weight basis of the substrate. The mushrooms were grown at the Mushroom Production Unit in the Faculty of Agriculture at Ahi Evran University, Kırşehir (Turkey). The production experiment was carried out in a completely randomized plot design with ten replicates for each growing medium.

Mushroom cultivation and evaluation of the cultivation parameters

The incubation period was conducted in air at 25±2°C and 70–80% relative humidity. When the spawn run was completed, the bags were transferred to a cropping room at 18±2°C and 80–90% relative humidity for induction of fructification and the cotton plugs at the top of the plastic bags were removed.Light was provided for 12 h daily using fluorescent bulbs. The ventilation was adequate to maintain the CO_2 concentration below 1000 ppm [18]. Mushrooms were harvested when they reached their full size.

During the cultivation period of *H. ulmarius* on different growing media, the study evaluated the spawn running period (SRP) (days), time to first primordia initiation (DPI) (days), time to first harvest (DFH) (days), and yield, as total fruit body weight (g)/total substrate weight (kg). The biological efficiency (BE), i.e., the total weight of fresh fruit bodies/dry weight of the substrate, was expressed as a percentage.

Substrate and mushroom analyses

Substrate samples to be analyzed were collected randomly from the experimental treatments after the sterilization period. After the mushroom and substrate samples were dried in an oven at 60 °C to a constant weight, they were stored at 4 °C until analysis. The ash content of the substrates was determined by following the standard procedure [19] Total carbon (C) was calculated from the ash [20]. Total nitrogen (N) was determined by the Kjeldahl method and the C:N ratios were calculated for the growing media.

The percentage of dry matter was calculated as the difference in the pre–and post-dry weights. The chemical composition of the *H. ulmarius* carpophore, including fat and ash contents, was determined according to AOAC procedures [19]. The fat content of the samples was estimated by extracting a known weight of powdered mushroom sample using a Soxhlet apparatus with petroleum ether as a solvent. The Kjeldhal method was used to determine the total N content of the fruitbody. The N content was then multiplied by a factor of 4.38 for the calculation of the crude protein in the mushrooms [21]. The total carbohydrate by difference was calculated [22], and the following equation of Heleno and coauthors [22] was used to calculate total energy; Energy (kcal) = 4 (g protein + g carbohydrate) + 9 (g fat)

The total phenolic content (TPC) of the growing media and mushrooms was measured according to Doğan and coauthors [23]. The results were expressed as mg of gallic acid equivalents (GAE) per g of dry carpophore of *H. ulmarius*. All analyses were conducted in triplicate in the Agriculture Faculty Laboratories of Ahi Evran University, Kırşehir, Turkey.

Statistical analysis

The results were statistically analyzed using the analysis of variance (ANOVA). Tukey's post-hoc comparison (at a significance level of 5%) was applied to determine individual differences between the means. All analyses were performed using SPSS 16.0 version

RESULTS

Chemical analyses of growing media

The chemical properties of the growing media are summarized in Table 2. The pH, ash (%), C (%), N (%), the C:N ratios and total phenolic content (mg GAE/g dw) were tested and in general, a high variability and statistically significant differences were observed (*p<0.01*).

Table 2. Proximate analysis and total phenolic content of growing media tested in the study.

Growing Media	pH	Ash (%)	C (%)	N (%)	C:N	Total Phenolic Content (mg GAE/g dw)
Control	6.68±0.15**c	6.67±0.37**a	54.1±0.22**d	1.17±0.11**a	46.6±4.2**d	1.27±0.09**g
WS:GWH10	7.03±0.21 bc	6.98±0.31 a	54.0±0.18 d	0.85±0.10 bc	64.5±8.2 d	2.38±0.11 f
WS:GWH20	7.34±0.10 ab	6.97±0.39 a	54.0±0.23 d	0.90±0.12 bc	62.0±8.7 d	3.31±0.21 d
WS:GWH30	7.32±0.19 ab	6.78±0.24 a	54.0±0.14 d	0.90±0.11 bc	60.6±8.6 d	4.17±0.12 bc
WS:GP10	6.68±0.17 c	6.95±0.23 a	54.0±0.13 d	0.97±0.06 ab	55.7±3.4 d	1.39±0.14 g
WS:GP20	7.23±0.09 ab	7.00±0.25 a	53.9±0.14 d	0.98±0.01 ab	54.8±0.8 d	4.58±0.15 b
WS:GP30	7.57±0.10 a	7.13±0.23 a	53.9±0.13 d	1.07±0.05 ab	50.3±2.2 d	6.35±0.21 a
PS:GWH10	5.25±0.14 e	2.39±0.13 d	56.6±0.07 a	0.51±0.07 de	112.9±14.5 ab	1.05±0.07 g
PS:GWH20	5.58±0.14 de	2.83±0.17 bcd	56.4±0.10 ab	0.41±0.06 e	135.2±18.8 a	2.92±0.22 de
PS:GWH30	6.01±0.12 d	3.26±0.18 bc	56.1±0.11 bc	0.48±0.05 de	120.8±10.9 ab	3.81±0.19 c
PS:GP10	5.35±0.17 e	2.60±0.07 cd	56.5±0.04 ab	0.43±0.07 e	135.5±20.9 a	2.60±0.13 ef
PS:GP20	5.84±0.11 d	3.16±0.12 bc	56.2±0.07 bc	0.58±0.06 de	98.7±10.1 bc	4.18±0.11 bc
PS:GP30	5.85±0.17 d	3.58±0.16 b	56.0±0.10 c	0.71±0.04 cd	80.4±4.5 cd	6.05±0.18 a

Each value is expressed as mean ± standard deviation (n=3).Asterisks indicate significance at *P<0.05,**P<0.01, ns not significant; values within the same column followed by the same letter are not significantly different by Tukey's test

The initial pH for the growing media was ranged between 5.25 (PS:GWH10) and 7.57 (WS:30GP). pH values of WS-based media were higher than PS-based media. The control substrate containing WS and WB showed the highest N content (1.17%), followed by the WS-based media supplemented by different ratios of GP (0.97–1.07%). The ash content of the growing media varied between 2.39% (PS:GP30) and 7.13% (WS:GWH10). C and C:N ratio varied considerably among the thirteen growing media and were found in the range of 53.9- 56.5% and 135.5-46.6, respectively. The phenolic content of the growing media supplemented with GP was higher than that of the others, with % 1.39-6.35 in WS based media and, 2.60-6.05% in PS based media.

Effect of phenolic rich agro-waste supplementation of growing media on cropping cycle of *H. ulmarius*

In the study, the differences between the control substrate and the experimental substrates were significant (p<0.01) during the growing period (SRP, DPI, and DFH).

The entire cropping period lasted 90 days. The *H. ulmarius* completed incubation within 20.2–31.2 days in the growing media examined. The PS:GWH10 and PS:GP20 media exhibited significantly shorter durations, 20.3 days and 20.2 days, respectively, than the other growing media tested. By presenting a longer SRP, the PS:GWH30 was the worst performing growing medium. The pinhead formation varied per substrate, taking 45.8–54.0 days on growing media prepared with WS and 44.2–55.3 days for those prepared with PS. In all growing media, the *H. ulmarius* fruitbody was harvested between 49.5-63th days of growing, approximately 5–6 days after pinheads appeared.

It is noteworthy that with PS:GWH10 was significantly faster than with the rest of the media evaluated. An increase in the length of the *H. ulmarius* cultivation period was observed for the PS:GWH30 and WS:GWH20 media compared with the other media.

Effect of phenolic rich agro-waste supplementation of growing media on yield parameters

H. ulmarius grew successfully in all substrates tested in the present study, as shown in Table 3. There were significant difference for total yield and BE(%) between the growing media (*P < 0.01*).

Total yields ranged from 161.1 g kg^{-1} to 235.4 g kg^{-1} in WS-based media and 58.5 g kg^{-1} to 133.7.0 g kg^{-1} in PS-based growing media, whereas the corresponding BE value ranges were 44.8–73.5% and 18.3–44.6%. The highest yield and BE (%) values were obtained from the WS:GP10 and WS:GP:20 media. The results of the study revealed that the addition of 10 and 20% ratios of GP significantly increased the productivity of the WS-based media by attaining BE values of 73.5 and 65.3%. The 20% GP addition to the PS-based substrate resulted in relatively high biological activity (133.7 g kg^{-1} and 44.6% BE), whereas the GP added at a 30% ratio to the growing medium significantly reduced the yield (58.5 g kg^{-1} and 18.3% BE). The number of flushes obtained from the growing media ranged from one to three. Although three flushes were obtained in both basal substrates supplemented with different ratios of GP (except for the PS:GP30 medium), two flushes were obtained from the media supplemented with GWH. In the three-flush harvested growing media, approximately 39–65% of the yield was obtained from the first flush, whereas the third flush contributed to 5.4–31.6% of the yield.

Assessment of nutritional composition and total phenolic content of *H. ulmarius* carpophores

Significant differences (*p<0.01*) were noticed in the ash, protein,fat, total carbohydrate contents, energy values and total phenolic contents of the carpophores, although the dry matter content not affected (*p>0.05*). The nutritional content of the carpophores revealed that the differences in their values depended on the nature of the growing media (Table 4).

Dry matter content varied considerably among those in the thirteen growing media and was found in the range of 7.93 –8.90%. The relatively wide variation in protein content obtained in the present study from the sawdust-based growing media (13.12–17.00%) and the wheat straw-based media (12.85–15.45%) Addition of the GWH significantly increased the fat content of the WS:GWH30 and PS:GWH30 fruitbodies, with WS:GWH30 showing the highest fat content (3.50%), followed by PS:GWH30 (3.21%). The lowest fat content (1.70%) was recorded in the fruitbodies harvested from WS:GP10. The ash content of the *H. ulmarius* fruitbodies, which ranged from 7.94 to 11.90%. Supplementation with GP and GWH caused a significant increase in ash content compared to the control (WS).

The total carbohydrates content in *H. ulmarius* ranged from 66.54% (WS:GWH30) to 75.15% (WS:GP10). Compared to the other media, the highest carbohydrate contents (75.15% and 74.55%) were found in WS:GP10 and WS:GP20. *H. ulmarius* was shown to be a poor source of energy, yielding 368.1–379.8 kcal 100 g^{-1} d.w.

The maximum TPC was determined in the fruitbodies grown on WS:GP30 (4.26 mg GAE g^{-1}) and WS:GP20 (4.14 mg GAE g^{-1}). Total phenols were 20% higher in fruitbodies harvested from WS:GP30 and WS:GP20 in comparison to the TPC of the control substrate harvest. Moreover, of the two different types of basal substrates, WS was more promising for the enhancement of the fruitbody phenolic content. High correlations were detected between phenolic content in the growing media and in the fruitbodies harvested ($r^2 = 0.791$, $p < 0.01$). This outcome was anticipated because of the properties of GP and GWH.

Table 3. Effect of different growing media on cultivation cycle and yield of *Hypsizygus ulmarius.*

Growing Media	Spawn Run Period (days)	Days to Pinhead Initiation (days)	Days to First Harvest (days)	Yield (g/kg)			Total Yield (g/kg)	Biological Efficiency (%)
				Flush 1 (g/kg)	Flush 2 (g/kg)	Flush 3 (g/kg)		
Control	29.0±1.4**bc	45.8±3.2** e	51.8±3.3** ef	101.5	72.7	51.5	225.7±6.2**a	64.5±1.8**b
WS:GWH10	28.0±1.4 c	52.0±20 bc	58.2±1.8 bc	85.4	64.8	0.0	161.1±8.5 c	44.8±2.6 de
WS:GWH20	30.5±2.3 ab	54.0±2.9 ab	59.5±2.6 b	106.7	62.0	0.0	168.7±13.2 c	45.6±3.6 d
WS:GWH30	25.0±1.3 d	49.7±1.7 cd	55.8±1.3 cd	102.9	73.5	0.0	166.7±8.4 c	46.3±2.3 d
WS:GP10	23.0±0.8 d	45.8±0.7 e	51.3±0.9 ef	103.4	77.1	54.7	235.4±12.1 a	73.5±3.8 a
WS:GP20	23.3±1.1 d	47.2±0.9 de	53.7±0.7 de	102.8	67.6	51.5	221.9±5.7 a	65.3±1.7 b
WS:GP30	27.8±1.2 c	53.0±1.5 ab	59.0±1.2 bc	73.7	56.8	60.0	190.5±6.0 b	57.7±1.8 c
PS:GWH10	20.3±0.7 e	44.2±0.7 e	49.5±1.1 f	70.3	38.9	20.5	129.7±10.2 de	39.3±3.1 f
PS:GWH20	27.5±0.5 c	51.8±0.7 bc	57.7±1.1 bc	81.8	47.9	0.0	129.7±7.7 de	40.5±2.4 def
PS:GWH30	31.2±1.1 a	55.3±1.6 a	60.8±2.0 ab	79.9	42.8	0.0	122.7±7.2 de	37.2±2.2 f
PS:GP10	27.7±0.7 c	52.5±1.0 abc	57.7±0.7 bc	61.0	48.5	6.2	115.7±3.9 e	37.3±1.3 f
PS:GP20	20.2±0.4 e	46.0±0.6 e	50.7±0.5 ef	55.6	44.3	33.8	133.7±5.0 d	44.6±1.7 de
PS:GP30	25.0±0.6 d	51.3±0.7 bc	63.0±1.3 a	58.5	0.0	0.0	58.5±7.4 f	18.3±2.3 g

Each value is expressed as mean ± standard deviation (n=10).Asterisks indicate significance at *P<0.05,**P<0.01, ns not significant; values within the same column followed by the same letter are not significantly different by Tukey's test

Table 4. Nutritional content and total phenolic content of *Hypsizygus ulmarius* fruitbody grown on different growing media.

Growing Media	Total Phenol (mg GAE/g)	Dry Matter (%)	Protein (%)	Fat (%)	Ash (%)	Total Carbonhydrate (%)	Energy (kcal)
WS:WB20	3.35±0.11**d	8.62±0.37 ns	19.34±0.61** b	2.16±0.01**f	7.94±0.02** g	70.51±0.60**d	379.1±0.1** a
WS:GWH10	3.53±0.15 bcd	8.32±0.51	12.85±0.03 g	2.05±0.05 f	10.72±0.01 bc	74.39±0.03 ab	367.4±0.3 e
WS:GWH20	3.70±0.06 bcd	8.10±0.25	14.87±0.07 de	3.10±0.07 bc	10.50±0.04 c	71.53±0.07 c	373.5±0.2 bcd
WS:GWH30	4.14±0.12 a	8.22±0.28	15.45±0.36 a	3.50±0.01 a	9.66±0.01 de	66.54±0.08 e	378.9±0.1 a
WS:GP10	2.65±0.20 f	8.18±0.49	13.11±0.09 fg	1.70±0.01 g	10.04±0.03 d	75.15±0.10 a	368.3±0.1 e
WS:GP20	3.94±0.06 ab	8.40±0.10	13.27±0.02 fg	2.60±0.01 e	9.58±0.12 e	74.55±0.13 a	374.7±0.5 bc
WS:GP30	4.26±0.19 a	8.90±0.54	14.58±0.02 de	2.79±0.02 de	8.54±0.19 f	74.08±0.20 ab	379.8±0.7 a
PS:GWH10	3.41±0.11 cd	8.41±0.27	13.28±0.04 fg	2.89±0.10 cd	9.69±0.29 de	73.42±0.17 b	375.7±1.5 b
PS:GWH20	3.52±0.19 bcd	7.87±0.19	14.00±0.11 ef	2.89±0.25 cd	10.41±0.08 c	70.70±0.72 c	372.8±1.4 cd
PS:GWH30	3.24±0.08 de	8.46±0.45	16.00±0.64 c	3.21±0.15 b	11.90±0.02 a	71.83±1.24 c	368.1±6.1 e
PS:GP10	2.92±0.15 ef	7.93±0.30	13.12±0.05 fg	2.25±0.02 f	9.44±0.22 e	73.36±0.67 b	373.5±0.9 bcd
PS:GP20	3.41±0.14 cd	8.14±0.29	14.95±0.51 d	2.27±0.01 f	9.78±0.03 de	71.26±0.15 c	372.2±0.1 d
PS:GP30	3.86±0.12 abc	8.54±0.53	17.00±0.16 a	3.16±0.03 bc	10.99±0.03 b	69.11±0.08 d	372.1±0.4 d

Each value is expressed as mean ± standard deviation (n=3).Asterisks indicate significance at *P<0.05,**P<0.01, ns not significant; values within the same column followed by the same letter are not significantly different by Tukey's test

DISCUSSION

The physical and chemical properties of the growing media are effective on the mycelial growth and yield of mushrooms. Fungal mycelium generally grows well at slightly acidic or near neutral pH [24]. The pH values of the growing media tested in the present study are between these values. The concentration of C and N content of substrate affect C:N ratio. Azizi and coauthors [25] reported that the N content in most of the substrates employed for mushroom cultivation ranged between 0.5 and 0.8%. Both the sawdust–and straw-based media supplemented with GP were rich in N and the other growing media were in the range recommended by Azizi and coauthors [25] Although the C:N ratios of the WS-based media were low compared with the higher N content of WS, the C:N ratios of the sawdust-based growing media examined were higher than the optimum values reported by Zied and coauthors [26]. Moreover, generally, the TPC levels of the growing media supplemented with GP were higher than in the rest of the growing media tested.

The shortened cultivation cycle is an important factor in the selection of growing substrates for mushroom production. In the study, PS: GWH10 and PS:GP20 media exhibited significantly shorter SPR than the other growing media tested. By presenting a longer SRP, the PS: GWH30 was the worst performing growing medium. The SRP values obtained in this study were similar or longer compared to those cited by Kumar and coauthors [13], Munna and coauthors [14], Sethi and coauthors [27], and Khade and coauthors [28] Although, an inhibitory effect of agricultural residues rich in phenolic content on the mycelial growth of mushroom species has been reported in previous studies [29,30], low ratios of GP and GWH in the growing media had no negative effect on the growth of *H. ulmarius* mycelium. Moreover, increased concentrations of GP, even at ratios of 20–30%, supported the growth of *H. ulmarius* mycelia and the cropping cycle was completed in a shorter time than with the control medium.

Statistical comparisons among the different growing media demonstrated that WS: WB, WS:GP10, PS: GWH10, and PS:GP20 were the most satisfactory media for fast pinhead formation in *H. ulmarius*, whereas PS: GWH30 provided the poorest results for the mushroom.

It is noteworthy that with PS: GWH10, mushroom production was completed within 49.5 days from inoculation, which was significantly faster than with the rest of the media evaluated. An increase in the length of the *H. ulmarius* cultivation period was observed for the PS: GWH30 and WS: GWH20 media compared with the other media. The DPI and DFH in the present study seemed to take considerably more time than in the study of Munna and coauthors [14], who reported that the appearance of *H. ulmarius* pinheads took 21.33–27.33 days, and first harvest started after 24.67–31.33 days. Differences in the DPI and DFH of a given substrate can be attributed to substrate type as well as to the fungal strain and growth conditions [24]

H. ulmarius grew successfully in all growing media supplemented with different ratios of GWH and GP. Moreover the mushroom did not form fruitbodies with deformities or abnormal shapes on the growing media supplemented with phenolic rich substrates. The straw-based substrates supported higher yields with both tested supplementation materials than the PS-based media. The higher nitrogen contents of the straw-based growing media may facilitate mushroom production by enhancing the activity of enzymes such as laccase and Mn-dependent peroxidase [31] or laccase and carboxymethyl cellulose [32]. Total yields obtained in the study fall within the values mentioned in previous studies [13,14,27,28] and some are higher than those reported by Rajini and Padmavadhi [33]. However, these values were lower than the results of Hausiku and Mupambwa [16] who reported that the BE of *H. ulmarius* was between 81 and 103% in different substrates.

The results of the study revealed that the addition of 10 and 20% ratios of GP significantly increased the productivity of the WS-based media. This could be related to the high ratios of several macro–and micronutrients in the GP [34]. According to the results, even the yield at a 30% ratio of GP supplementation (WS:GP30) was not much lower than that of the control. On the other hand, although the addition of 30% GP to the straw-based media led to a slightly decrease in yield and productivity, these parameters decreased much more drastically in the PS-based media. Presumably, the presence of a high phenolic content in the growing medium supplemented with 30% GP may have played a critical role in decreasing the yield and BE by inhibiting mushroom growth and the biodegradation process via inhibition of the lignocellulosic enzymes. Several researchers added olive mill wastes rich in phenolic content to the growing medium and reported that, comparable to the outcome of this study, a 20% ratio was the optimum concentration for effective phenolic biodegradation by white rot fungi [5,35]. Moreover, previous studies have reported that the use of agricultural wastes rich in phenolic content such as olive press cake [36] or olive press effluent [5] in high ratios decreased *Pleurotus* spp. mushroom production.

The low BE value of the sawdust-based media could be explained by the relatively high C:N and lignin content, and low hemicellulose content of sawdust [26,37]. However, assessment of the productivity of growing media is not an easy task since mushroom yield and earliness depend also on other factors such as

nutritional content, toxic constituents (e.g., juglone and phenolics), interactions among lignocellulosic components, physical properties of the substrates (e.g., compact texture), and more.

The straw–and sawdust-based growing media supplemented with GWH underperformed compared to the control. Stampar and coauthors [38] reported the presence of a high phenolic constituent called juglone in GWH. The juglone content of the GWH may have been the most important cause of lower *H. ulmarius* yield, despite the growing media having close to ideal values in terms of lignocellulosic content and N. Juglone is an example of an allelopathic compound that is synthesized by walnut and affects the growth of other plants [39,40] However, increases in the supplementation levels of GWH in the growing media did not affect the yield. It is of paramount importance that these results are achieved by adding supplements rich in phenolic content that are reported to be harmful to field and water resources. Although the supplemental materials widely used in mushroom cultivation such as wheat bran and soybean flour provide easily assimilated nutritional forms to mushroom mycelium, they are also more expensive and are widely used for other purposes. Therefore, the use of materials such as GP and GWH as supplement material in the preparation of the growing media instead would be beneficial in terms of reducing production costs and protecting environmental health.

The nutritional content of the carpophores revealed that the differences in their values depended on the nature of the growing media. The thirteen evaluated growing media presented wide variations in *H. ulmarius* mushroom crude protein, fat, ash, carbohydrate, and energy. Dry matter content is within the intervals reported by Kalac [41] for mushrooms. Higher protein content was recorded in the control medium compared to the media supplemented with different ratios of GP and GWH. Moreover, the protein value of the fruitbodies was enhanced with an increase in the rates of GP and GWH. According to Wang and coauthors [42], not only the supplementation ratio, but also the properties of the N source added to the growing media affected the protein content of fruiting bodies. In addition, the supplementation of growing media with wheat bran showed greater potential for increasing the accumulation of protein in *P. ostreatus* [42]. The protein contents obtained in the present study were in accordance with the results obtained in previous studies [16,43]. Reports of significantly higher protein contents in *H. ulmarius* [14,44,45] are atypical and were seen to result from using 6.25 as the coefficient to calculate the mushroom protein content instead of 4.38, which should have been used [21]. The fat contents obtained in the present study were similar to those previously reported for *H. ulmarius* mushrooms grown on different substrates [14,43,44], whereas distinctly higher values were obtained in several pertinent studies [16,45]. Overall, *H. ulmarius* mushrooms produced on growing media supplemented with high ratios of GP and GWH exhibited significantly higher fat concentrations when compared to most of the other substrates examined. This may be related to the considerably high fat contents of GP and GWH (data not shown). Supplementation with GP and GWH caused a significant increase in ash content compared to the control (WS). In this study, the ash content values for *H. ulmarius* cultivated on different growing media were similar to those obtained from other studies [16,43,45]. The relatively wide variation in carbohydrate contents were obtained in the present study depends on the growing media. Compared to the other media, the highest carbohydrate contents were found in WS:GP10 and WS:GP20. Moreover, past studies have reported lower or similar carbohydrate values for some other mushroom species [46]. These variations between different studies may be attributed to the use of different methodologies, substrates, and strains. Peter [47] reported that fungal carbohydrates can be considered as dietary fiber, which facilitates the reduction of blood glucose levels along with other therapeutic actions. The finding energy values are similar to relevant literature values [22]. *H. ulmarius* mushroom is suitable for use in low-calorie diets due to its low fat and energy content.

Phenolic compounds are among the most widely distributed plant secondary metabolites and act as potent antioxidants. Literature data are limited concerning the total phenolic contents of cultivated *H. ulmarius* mushrooms [48,49]. Moreover, the present work assessed for the first time the TPC of *H. ulmarius* cultivated on different growing media supplemented with phenolic-rich agro-wastes. The TPC of *H. ulmarius* fruitbodies grown on different media varied between 2.65 and 4.26 mg GAE g^{-1} compared to the reported values in other mushroom species including *Lentinula edodes* (18.03 mg GAE g^{-1}) [50], *Ganoderma lucidum* (2.107 mg GAE g^{-1}), *Pleurotus ostreatus* (36.0 mg GAE g^{-1}) [51], *Hericium erinaceus* mycelium and fruitbodies (3.82–4.89 mg GAE g^{-1}) [52]. Generally, it seemed that increases in TPC concentration were found to be significantly associated with higher ratios of GP and GWH in the growing media. This effect was extremely noticeable for the 30% GP supplementation. It is notable that supplementation with higher ratios of GP resulted in a consistent increase in the fruitbody TPC, and that the use of different ratios of GWH also led to high TPC in the mushrooms. Moreover, the detected correlations associating mushroom phenolic content with the phenolic content of the growing media are in agreement with previous findings with other mushroom species [7-9], thus highlighting the importance of the presence of such components in the cultivation substrates. Such

results indicated a potential to enhance mushroom some functional and nutritional value through the suitable selection or modification of the cultivation substrates.

CONCLUSION

This study presented for the first time information on the effects of these high-phenolic materials on the yield, productivity, bioactivity, and nutrient content of *H. ulmarius* and reported the emerging positive results related to its use. The WS-based media supplemented with different ratios of GP demonstrated similar or better yields and productivity values for *H. ulmarius* when compared to the traditional substrate (control). On the other hand, the growing media with high ratios of GP resulted in *H. ulmarius* fruitbodies with significantly richer phenolic contents. Polyphenol-rich agro-food wastes could also be suitable for replacing conventional wheat bran supplementation material as an alternative supplement material because it is readily accessible and available in large amounts, especially in walnut and grape producing countries. However, further studies are needed to gain in-depth information about the possible effects of fungal enzymes on the juglone content and on other polyphenolics in the spent mushroom substrates.

Conflicts of Interest: The authors declare no conflict of interest.

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