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The effect of some agro– industrial wastes on yield, nutritional characteristics and antioxidant activities of *Hericium erinaceus* isolates

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ABSTRACT

Four isolates of *Hericium erinaceus* cultivated in different growing media were investigated for their mycelial growth, yield, biological efficiency (BE), macro and micro element content, total phenolic content and antioxidant activity. In the study, oak sawdust (OS) was used as a base substrate, and cottonseed hulls (CSH) and olive press cake (OPC) were added at the ratios of 9:1, 8:2 and 7:3 to prepare the growing media. The control medium was prepared using OS and wheat bran (WB) at the rate of 8:2. The spawn run-period was shorter in all *H. erinaceus* isolates growing in the OS:WB (control) medium. The yield and BE (%) of *H. erinaceus isolates* ranged between 76.7 and 152.9 g/kg and 22.3–44.4%, respectively, depending on the growing medium used. The highest yield and BE% for all *H. erinaceus* isolates, except He-Trabzon, was obtained on 7OS:3CSH medium. The nutritional composition of *H. erinaceus* isolates varied with the growing medium, but there was no direct relationship between the macro- and micro-element content of the growing media and the nutrient content of the fruitbodies. The antioxidant activity and phenolic content of *H. erinaceus* isolates grown on different growing media ranged between 1.76 and 4.92 µmol TE/g fw and 0.318–0.663 mg GAE/g fw, respectively. The antioxidant activity and phenolic content of He- Ankara, He-Denizli and He-Trabzon were not affected by the growing media, whereas the addition of OPC to the oak sawdust substrate had a noticeable effect on the phenolic content and antioxidant activity of the fruitbodies of HE-İzmit. According to the results, cotton seed hulls and olive pess cake can be recommended as alternative additive materials to wheat bran to increase the yield of *H. erinaceus*. Finally, the use of olive press cake as substrate incrases the phenolic content of *H. erinaceus* mushrooms.

1. Introduction

Mushrooms have been collected and consumed by people for centuries. They are a healthy food, low in calories, and high in degradable proteins, iron, zinc, chitin, fibre, vitamins and minerals (Manzi et al., 2001; Vetter, 2007; Reis et al., 2012). Beyond their nutritional characteristics, mushrooms have been reported to posses medicinal properties (Roncero-Ramos and Delgado-Andrade, 2017; Muszynska et al., 2018).

Hericium erinaceus is a species belonging to the class *Agaricomycetes*, the order *Russulales* and the family *Hericiaceae*. It is popularly known as lion's mane, monkey's head, hedgehog fungus, pom pom mushroom and yamabushitake. *H. erinaceus* is an edible mushroom with an excellent flavour and nutritional value (Imtiaj et al., 2008; Friedman, 2015). Moreover, the medicinal properties of *H. erinaceus* have been well known for hundreds of years in traditional Chinese and Japanese medicine (Wang et al., 2014). Also today, the extract of *H. erinaceus* is reported to exhibit antimicrobial, anticancer (Gue et al., 2006), antitumor (Park et al., 2002), and blood lipid-lowering (Keun et al., 2003) properties and is used in the prevention and treatment of some cancers such as those of the oesophagus, stomach, and skin (Mizuno, 1999). *H. erinaceus* is rarely recorded in Europe (Boddy et al., 2011). The *H. erinaceus* mushroom was first cultivated in the 1960s, and today it is widely produced in many countries as both an edible and a medicinal mushroom.

Turkey has a large edible mushroom potential and is becoming an important exporter of wild mushrooms. One of the mushroom species that grows in Turkey naturally is *H. erinaceus*. Wild samples of *H. erinaceus* have been collected in Sinop (Afyon et al., 2004), the Black Sea Region of Turkey (Afyon et al., 2005) and Istanbul (Akata, 2017), but

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Table 1
Macro and micro element content of different growing media used in the study.

Substrate	Ash (%)	C (%)	N (%)	C:N	P (g kg^{-1})	K (g kg^{-1})	Ca (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
OS:WB	6.4 ± 0.08^{**bc}	46.8 ± 0.04^{**bc}	0.68 ± 0.01$^{** b}$	68.8 ± 0.39$^{** c}$	0.21 ± 0.02$^{** c}$	0.32 ± 0.01$^{** a}$	2.6 ± 0.09$^{** c}$	0.11 ± 0.001$^{** b}$	0.02 ± 0.002$^{** c}$	119.4 ± 3.21$^{** e}$	53.5 ± 1.82$^{** c}$	78.8 ± 0.76$^{** a}$	8.9 ± 0.20$^{** d}$
9OS:1CSH	5.4 ± 0.12^{c}	47.3 ± 0.06^{ab}	0.57 ± 0.01^{c}	82.7 ± 0.82^{c}	0.29 ± 0.04^{b}	0.28 ± 0.01^{b}	3.1 ± 0.07^{b}	0.09 ± 0.012^{c}	0.02 ± 0.003^{c}	288.1 ± 7.38^{b}	53.5 ± 0.98^{c}	61.2 ± 0.67^{b}	8.7 ± 0.43^{d}
8OS:2CHS	6.2 ± 1.19^{bc}	46.9 ± 0.60^{abc}	0.60 ± 0.02^{c}	78.3 ± 2.81^{c}	0.30 ± 0.05^{b}	0.27 ± 0.03^{b}	3.4 ± 0.30^{b}	0.10 ± 0.001^{bc}	0.03 ± 0.001^{b}	284.9 ± 8.54^{b}	56.5 ± 0.45^{b}	58.0 ± 0.89^{c}	9.5 ± 0.32^{c}
7OS:3CHS	8.6 ± 0.12^{a}	45.7 ± 0.06^{d}	0.88 ± 0.04^{a}	51.7 ± 2.53^{d}	0.40 ± 0.02^{a}	0.27 ± 0.01^{b}	3.9 ± 0.06^{a}	0.13 ± 0.008^{a}	0.04 ± 0.004^{a}	330.4 ± 3.91^{a}	63.1 ± 0.42^{a}	61.1 ± 2.49^{b}	11.8 ± 0.81^{ab}
9OS:1OPC	5.8 ± 0.15^{bc}	47.1 ± 0.07^{abc}	0.26 ± 0.02^{e}	178.8 ± 15.73^{a}	0.11 ± 0.01^{d}	0.17 ± 0.01^{d}	1.9 ± 0.07^{d}	0.04 ± 0.001^{d}	0.01 ± 0.001^{d}	133.7 ± 2.43^{e}	48.3 ± 1.52^{d}	54.8 ± 0.11^{d}	10.5 ± 0.84^{b}
8OS:2OPC	5.3 ± 0.12^{c}	47.3 ± 0.06^{a}	0.32 ± 0.01^{d}	147.1 ± 2.56^{b}	0.13 ± 0.02^{e}	0.22 ± 0.02^{c}	1.9 ± 0.08^{d}	0.04 ± 0.001^{d}	0.01 ± 0.001^{d}	151.4 ± 4.78^{d}	43.9 ± 1.82^{e}	51.6 ± 0.74^{e}	11.5 ± 0.74^{ab}
7OS:3OPC	6.5 ± 0.51^{b}	46.8 ± 0.25^{c}	0.33 ± 0.02^{d}	146.8 ± 10.57^{b}	0.14 ± 0.02^{e}	0.26 ± 0.03^{b}	1.8 ± 0.17^{d}	0.04 ± 0.002^{d}	0.01 ± 0.004^{d}	178.8 ± 5.02^{c}	35.4 ± 0.40^{f}	57.2 ± 3.40^{cd}	12.2 ± 0.79^{a}

n.s. – no significant; * – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$.Mean values in the same column followed by the same letters are not significantly different by Tukey's tests.

no information is available on their nutritional content and antioxidant properties.

For their part, olive press cake and cotton seed hulls are common by-products in the Mediterranean Basin, the latter a fibrous product primarily used to feed ruminants (Hall and Akinyode, 2000). However, olive press cake has limited use because of its low digestibility and energy content (Al-Masri and Guenther, 1999) and high content of phenolic compounds (Suárez et al., 2010). *H. erinaceus* has been reported to be easily grown on different types of lignocellulosic waste, such as beech and ash tree sawdust, wheat bran (Ehlers and Schnitzler, 2000), soybean flour (Siwulski and Sobieralski, 2005), rice bran, barley bran, soybean powder, egg shell, chinese cabbage (Ko et al., 2005), and sunflower hulls (Figlas et al., 2007). In previous studies with different fungal species, the growing medium was seen to influence the functional, organoleptic, and chemical properties of mushrooms such as *Pleurotus ostreatus* and *Hericium americanum* (Oyetayo and Ariyo, 2013; Yildiz et al.,1998; Atila et al., 2017).

Although some authors have demonstrated the presence of some macro- and microelements in *H. erinaceus* (Heleno et al., 2015), there has been no report about the effect of different growing media on the macro- and micro element content. Furthermore, many polysaccarides were isolated and identified by Mori et al. (2010) and Kim et al. (2011) from the fruitbody or mycelium of *H. erinaceus*. However, little is known about the antioxidant properties and total phenolic content (Wong et al., 2009; Han et al., 2013; Heleno et al., 2015; Koutrotsios et al., 2016).

For the above reasons, the objectives of this work were: (1) to determine suitable additive materials for the cultivation of *H. erinaceus* isolates; (2) to compare the proximate composition of isolates of *H. erinaceus* grown on different media; (3) to discover the relationship between the nutritional composition and their nutrient source; (4) to investigate the influence of the growing media composition on the total phenolic content and antioxidant activity of four isolates of *H. erinaceus*.

2. Materials and methods

2.1. Materials

H. erinaceus isolates collected from different areas of Turkey (Ankara, Denizli, Trabzon, İzmit) for a breeding project by a mushroom spawn company (Agroma Co. Ltd., Denizli, Turkey). Pure cultures of *H. erinaceus* isolates were supplied by the above-mentioned company and maintained on a malt extract agar (MEA) at 4 °C.

Wheat bran (WB) cottonseed hulls (CSH), and forest industry waste (oak sawdust, OS) were obtained from local markets in Izmir (Turkey). Olive press cake (OPC) was supplied by a pomace oil factory (Helvacikoy, Izmir, Turkey).

2.2. Growing media preparation

Oak sawdust was used as a base substrate, and cottonseed hulls and olive press cake were added in the ratios of 9:1, 8:2 and 7:3 to prepare the growing media. A control medium was prepared using OS (at the rate of 80%) and WB (at the rate of 20%) on a dry weight basis of the substrates (Table 2). The respective growing media were soaked with distilled water overnight, and the excess water was drained to reach a substrate moisture level of about 70%. Then, 1 kg (wet weight) of each growing medium was packed into a polypropylene autoclavable bag and autoclaved at 121 °C for 90 min.

2.3. Mushroom cultivation

After sterilization, the growing media were inoculated with 3% grain spawn (on a wet weight basis) and incubated at 25 ± 2 °C with 80% relative humidity in the presence of light for mycelial colonization. After full colonisation, the bags were transferred to a cropping room at

20 ± 2 °C, 80–90% RH with 8 h of light daily to induce fructification. Sufficient air changes were made to keep the CO_2 concentration below 1000 mg/kg^{-1}. Mushroom were harvested when the fruitbody spines reached 5 mm.

Mushrooms were grown at the Mushroom Production Unit and the Laboratories of the Horticulture Department in the Faculty of Agriculture at Ege University, Izmir (Turkey). The production experiment was carried out in a completely randomized plot design, with ten replications.

2.4. Evaluation of the cultivation parameters

Several cultivation parameters were evaluated during the cultivation period of *H. erinaceus* isolates on different growing media: spawn running time (day), time to first primordia initiation (day), time to first harvest (day), yield (g/kg), biological efficiency (BE%) and avarage mushroom weight (g). Total yield was calculated as the sum of two flushes and expressed as grams of fresh mushrooms harvested per gram of wet growing medium (w/w).

The biological efficiency (BE%), defined as the percentage ratio of the fresh weight of harvested mushroom to the dry weight of growing medium, was calculated using the formula;

BE (%) = (fresh weight of harvested mushroom per bag / dry weight of growing medium per bag) × 100

2.5. Elements analysis

Mushroom and growing medium samples were oven dried at 60 °C for 48 h. The dried materials were ground into powered form using a grinder. The obtained power was used for ash (%), total nitrogen (%) and macro– and micro element analysis.

The ash content was estimated from the samples incinerated in a muffle furnace at 550 °C for 16 h. The total nitrogen content of the samples was determined by the Duchaufour (1970) method. The crude protein content of the fruitbodies was calculated by using the adjusted conversion factor (N × 4.38). The ash residues were digested in 0.6 N nitric acid (HNO_3) and the obtained solution was used determination of nutrients by atomic absorption spectrometry (AAS) (AAnalyst 800 AAS, Perkin Elmer Inc.) (Madrid et al., 1996). Phosphorus (PO_4^{-3}) was measured by spectrophotometer (MAPYA, 1998).

Analysis of the macro- and microelements was carried out in the GARSA Laboratories at the Technical University of Cartagena (Spain), with three replications.

2.6. Total phenolic content and antioxidant activity

The spectrophotometric method with the ferric reducing antioxidant power (FRAP) was applied to antioxidant activity determination in *H. erinaceus* extracts (Benzie and Strain, 1996). Five grams of fruitbody were mixed with 25 mL of methanol and homogenised using an Ika Ultra-Turrax homogeniser. The homogenates were kept at 4 °C and in the dark for 14–16 h and then filtered using a Whatman No. 4 filter paper. The supernatants were recovered and stored at −20 °C until analysis (Thaipong et al., 2006). The absorbance of the supernatant was recorded using a spectrophotometer (Bio 100, Varian, Australia) at 593 nm. Trolox was used as a standard and the results are expressed as Trolox equivalents (μmol TE g^{-1} fresh weight).

The total phenolic content of the phenolic extract of *H. erinaceus* isolates was determined using the Folin-Ciocaltaeu method with some modifications (Swain and Hillis, 1959). The absorbance of the supernatant was recorded at 725 nm by a spectrophotometer (Bio 100, Varian, Australia). Gallic acid was used as a standard. The total phenolic content in the extract was expressed as gallic acid equivalents (GAE) in mg/g^{-1} fresh weight (fw).

The analysis of the total phenol content and antioxidant activity were conducted at the Laboratories of the Horticulture Department in the Faculty of Agriculture at Ege University in Izmir (Turkey), with three replications.

2.7. Statistical analysis

All statistical analyses were performed via SPSS 16.0 software. For the *H. erinaceus* isolates grown on different media, a two-way ANOVA was used to test the significance of the isolate and of the growing medium as well as their interaction on macro and micro element content, antioxidant activity, total phenolic content and on yield parameters. The significant interactions were further examined for the main substrate effects, i.e. the effect of each medium separately for each isolate. Differences between the means of individual groups were assessed via Tukey's test at a significance level of 5%. Pearson's correlation was used to analyse the relationship between the chemical constituents of the growing medium and the spawn running time, yield and macro- and microelement content of the fruitbodies.

3. Results and discussion

3.1. Effect of growing medium composition on mycelial growth and productivity parameters

The different growing media compositions led to considerable differences in the ash content, C:N ratio and content of N, P, K, Ca, Mg, Na, Fe, Mn, Zn, and Cu ($P < 0.01$), no statistically significant differences were found in the moisture content ($P > 0.05$) (Table 1).

The highest N, P, Mg, Ca, Na, Fe, Mn, and Cu contents were determined in the 7OS:3CSH, while the control medium exhibited the highest K and Zn contents. The lowest macro- and microelement contents were found in the growing media supplemented with OPC. The C:N ratios of the growing media varied between 68.8 and 178.8.

Significant differences were observed among treatments of *H. erinaceus* isolates in terms of days taken for spawn running time, time to first primordia initiation and time to first harvest ($P < 0.01$) (Table 2, Fig. 1).

The spawn running time of *H. erinaceus* isolates grown on media supplemented with different additives ranged from 27.0 to 38.3 days depending on the isolate evaluated and the growing medium. The incubation period appeared to be slightly a little shorter than the findings Hassan (2007), who reported that the spawn running time of *H. erinaceus* grown on sawdust ranged between 43 and 46 days. However, Stamets (1993) reported a spawn running time for *H. erinaceus* of 10_14 days at 21_24 °C, which is shorter than that determined in the present study. Spawn running time can vary with mushroom strain and growing substrates. The results of this study suggest that wheat bran is the most effective and suitable substrate for rapid and maximal mycelial running of *H. erinaceus* isolates. Chang and Roh (1999); Ehlers and Schnitzler (2000); Siwulski and Sobieralski (2005) also reported similar findings on the stimulating effect of wheat bran on mycelial growth of *H. erinaceus*. The mycelial growth of mushrooms depends on the nitrogen and macro and micro element content (Adenipekun and Gbolagade, 2006). The results of the study revealed that the C:N ratio of the medium also influenced spawn running time, earliness and crop cycle duration. The C:N ratio of the 7OS:3OPC medium, which led to the longest spawn running time, nearly two times higher than that of the control and 7OS:3CSH. The positive relation between spawn running period and C:N ratio could explain the lateness of fructification in the media having higher C:N ratios. Moreover, the first primordia also appeared later in these media. The negative effect of high concentrations of OPC on earliness in several mushroom species has also been reported in previous works (Gregory and Pohleven, 2014; Zervakis et al.,2013). This could be due to the high phenolic content of OPC (Aludatt et al., 2010), as well as the low nitrogen content of the substrate (Atila et al., 2017). In addition, the macro and micro element content and C:N ratio of the growing medium and the physical structure of the additive materials

Table 2
F test of the two-way ANOVA and its statistical significance (P) for isolate, substrate and their interaction in yield parameters of *Hericium erinaceus* isolates grown on different growing media.

		Spawn running time	Days to primordia initiation	Days to first harvest	Yield	Biological efficiency	Average mushroom weight
Isolate	*F*	38.6	308.9	737.7	147.7	65.2	161.7
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Growing medium	*F*	180.0	529.4	1259.7	67.7	30.5	74.2
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Isolate x growing medium	*F*	5.0	7.2	37.0	13.0	8.0	14.2
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

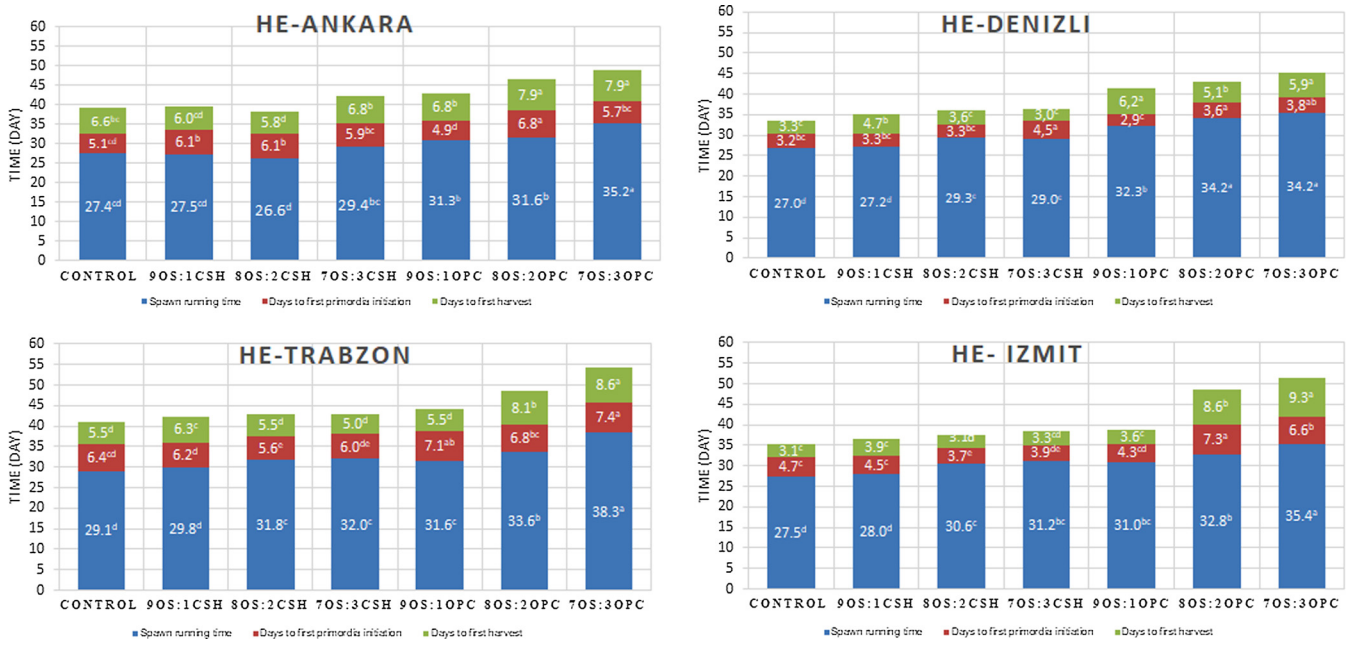

Fig. 1. Effect of growing media on production stages.

can also effect mycelial growth. The crucial role of wheat bran in rapid mycelial growth might be attributed to its physical properities such as bigger particle size, denser crumb texture and higher oxygen capacity. Gregory et al. (2012) suggested that in addition to the phenolic content of OPC, its low porosity could have a negative influence on mycelium growth and the production of fruiting bodies. He-Denizli was the earliest of all the isolate tested, forming their first primordia after 30.2_39.2 days and providing the first harvest after 33.5_45.1 days of incubation in various media. On the other hand, He-Trabzon was the slowest growing isolate with the first primordia formed after 35.5_ 45.7 days and the first harvest obtained after 41.0_54.3 days of incubation on the media used in the study.

The effect of the isolate, the growing medium and their interaction on the yield, BE and average mushroom weight was found to be statistically significant ($P < 0.01$). The total yield of *H. erinaceus* isolates varied between 66.2 g kg^{-1} and 152.9 g kg^{-1}, while the BE varied between 19.2% and 44.4% in the different growing media (Fig. 2). The yield and BE values determined in the study were similar to those obtained by Ko et al. (2005), Ehlers and Schnitzler (2000) and Siwulski and Sobieralski (2005). Interaction plots showed that He- Izmit gave the highest yield when supplemented with CSH at a 20 level %, followed by at a 30% level, while the lowest yield was observed at 30% OPC in He-Ankara. Generally, *H. erinaceus* isolates showed a positive linear response to increasing levels of CSH. This could be related to the higher macro and micro element content of the CSH substrate. Unlike the other isolate, He-Trabzon grown in 7OS:3OPC medium gave the highest yield and BE. These results showed that the substrate preference and yield capacity of *H. erinaceus* isolates are different, in order to achieve maximum productivity of *H. erinaceus* matching both strain and substrate is very important. For He-Trabzon and He-Izmit, the yields obtained using media to which OPC was added are promising. The 7OS:3OPC combination for He-Trabzon and He-Izmit gave 37.7% and 27.3% higher yields, respectively, than the control medium. On the other hand, when the growing medium was supplemented with OPC at different levels, He-Ankara and He-Denizli isolates reached maximum yield at the 10% level and then declined. Thus, it is possible that each isolate has a different levels of sensitivity to the phenolic content in the growing medium.

The average mushroom weight ranged between 39.5 and 76.4 g in the *H. erinaceus* isolates grown in the different media. The 70S:3OPC and 8OS:2OPC media led to the highest average mushroom weight, with He-Izmit achieving a substantially higher average mushroom weight than the other isolates. The isolate x growing media interactions were significant for average mushroom weight. In the present case, the highest fruitbody weight in the study was produced by He-Izmit on substrates containing CSH at 20% and 30% levels. A correlation between yield and average mushroom weight is a expected result. Consequently, the highest average mushroom weight observed in the He-Ankara and He-Denizli isolates was for those grown on the media in which the lowest yield was obtained. In light of these results, the yields

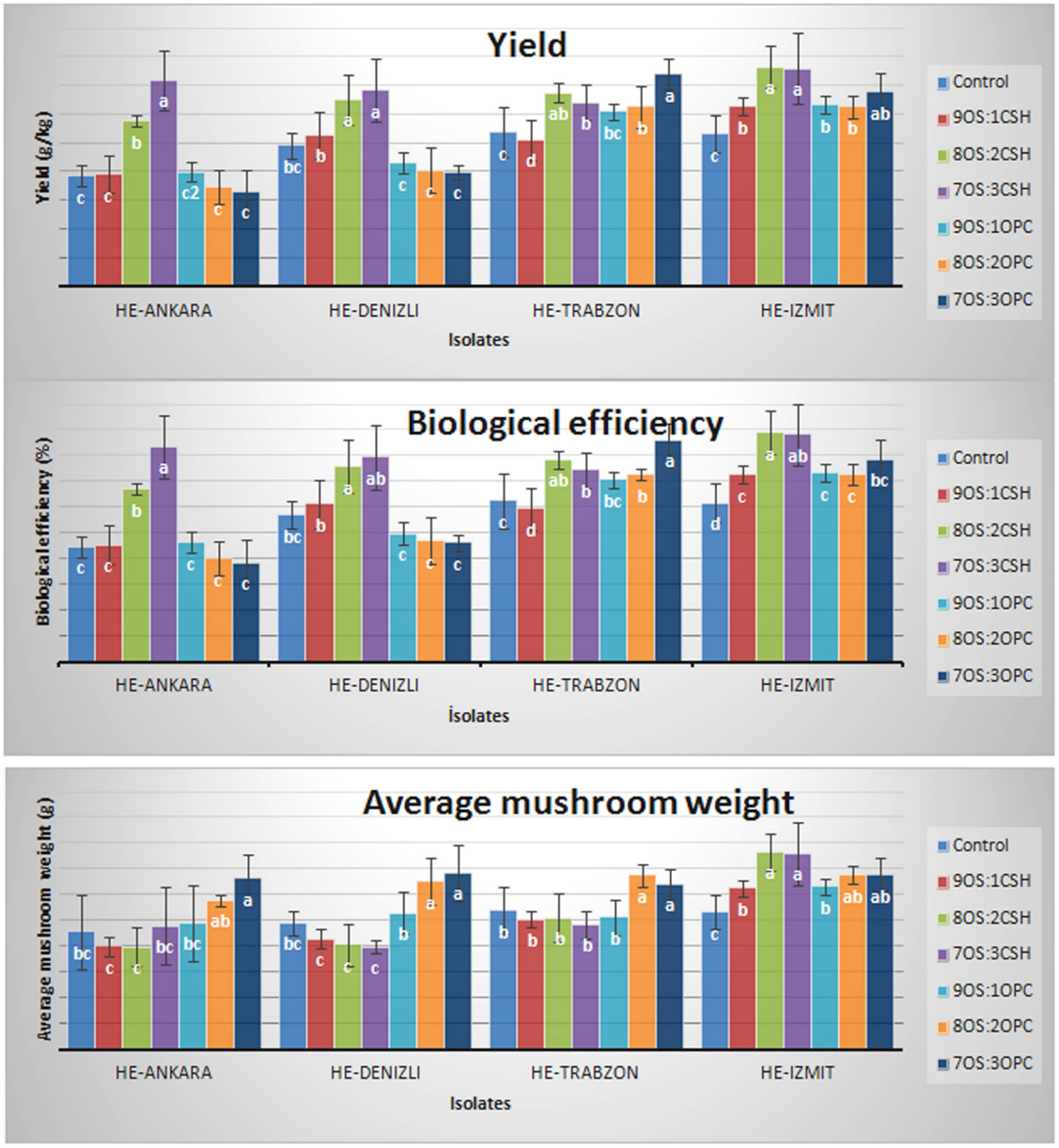

Fig. 2. Effect of different growing media on productivity.

Table 3
F test of the two-way ANOVA and its statistical significance (P) for isolate, substrate and their interaction in macro and micro element content, antioxidant activity and total phenolic content of *Hericium erinaceus* fruitboy grown on different growing media.

		Ash	Protein	P	K	Mg	Ca	Na	Fe	Mn	Zn	Cu	Antioxidant activity	Total phenolic content
Isolates	*F*	8.5	94.4	122.9	158.3	34.8	15.1	122.8	38.0	275.3	84.4	11.6	57.96	54.20
	P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Growing medum	*F*	3.9	274.3	469.6	70.8	59.6	33.6	11.7	102.3	39.8	46.2	2.4	2.562	2.11
	P	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.41	0.029	0.067
Isolatexgrowing medium	*F*	0.5	12.7	40.5	12.9	20.3	13.3	27.6	19.4	10.6	11.3	1.7	2.10	1.72
	P	0.958	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.60	0.018	0.064

of He-Ankara and He-Denizli could have been sensitive to the phenolic content of OPC. Because of this sensitivity, fewer pinheads were formed in the growing media supllemented OPC, resulting in larger and heavier fruitbodies.

3.2. Effect of different growing media on the chemical composition of fruitbodies

In the present study, the ash, N, P, K Ca, Mg, Na contents and the microelements Fe, Cu, Mn, and Zn were calculated in the four *H. erinaceus* isolates grown in seven different growing media. The isolate, the

Table 4
Macro element composition of fruitbody of *Hericium erinaceus* isolates grown on different growing media.

İsolates	Substrates	Ash (%)	Protein (%)	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca(g kg^{-1})	Na (g kg^{-1})
He-Ankara	Control	8.50 ± 0.48^{ns}	20.04 ± 0.72$^{a\ **}$	28.40 ± 0.84$^{a\ **}$	38.98 ± 2.54$^{a\ **}$	1.03 ± 0.01$^{ab\ **}$	0.24 ± 0.00^{b**}	0.143 ± 0.01^{c**}
	9OS:1CHS	8.21 ± 0.31	15.05 ± 0.03^{c}	23.80 ± 0.09^{b}	26.65 ± 3.03^{c}	0.88 ± 0.10^{c}	0.28 ± 0.02^{a}	0.138 ± 0.00^{c}
	8OS:2CHS	8.17 ± 0.55	14.13 ± 0.01^{c}	22.61 ± 0.23^{b}	31.27 ± 1.59^{bc}	0.93 ± 0.01^{bc}	0.24 ± 0.02^{b}	0.199 ± 0.01^{b}
	7OS:3CHS	8.29 ± 0.64	16.78 ± 0.42^{b}	23.39 ± 0.22^{b}	31.54 ± 0.90^{b}	1.07 ± 0.02^{a}	0.24 ± 0.01^{ab}	0.270 ± 0.00^{a}
	9OS:1OPC	7.96 ± 0.63	12.23 ± 0.34^{d}	13.73 ± 0.56^{d}	30.64 ± 0.38^{bc}	0.95 ± 0.01^{bc}	0.22 ± 0.01^{b}	0.198 ± 0.01^{b}
	8OS:2OPC	7.92 ± 0.46	12.45 ± 0.34^{d}	16.73 ± 0.26^{c}	32.36 ± 0.21^{b}	0.96 ± 0.00^{abc}	0.23 ± 0.00^{b}	0.186 ± 0.00^{b}
	7OS:3OPC	8.11 ± 0.52	12.50 ± 0.41^{d}	16.74 ± 0.28^{c}	35.03 ± 0.46^{ab}	0.96 ± 0.03^{abc}	0.23 ± 0.01^{b}	0.190 ± 0.01^{b}
	Mean	**8.16** ^{b}	**14.74** ^{a}	**20.77** ^{a}	**32.35** ^{ab}	**0.97** ^{a}	**0.239** ^{b}	**1.89** ^{b}
He-Denizli	Control	8.72 ± 0.28^{ns}	16.19 ± 0.94$^{a\ **}$	26.23 ± 0.15$^{a\ **}$	26.98 ± 0.52$^{a\ **}$	1.04 ± 0.05$^{a\ **}$	0.29 ± 0.01$^{b\ **}$	0.248 ± 0.01$^{b\ **}$
	9OS:1CHS	8.31 ± 0.23	11.83 ± 1.03^{b}	16.59 ± 0.36^{b}	19.38 ± 0.03^{c}	0.71 ± 0.01^{c}	0.22 ± 0.00^{c}	0.317 ± 0.01^{a}
	8OS:2CHS	8.33 ± 0.44	12.29 ± 0.95^{b}	16.56 ± 0.25^{b}	20.74 ± 0.72^{c}	1.11 ± 0.03^{a}	0.28 ± 0.01^{b}	0.161 ± 0.01^{cd}
	7OS:3CHS	8.22 ± 0.27	12.49 ± 0.36^{b}	15.68 ± 0.08^{b}	25.09 ± 0.12^{b}	0.85 ± 0.02^{b}	0.34 ± 0.01^{a}	0.132 ± 0.00^{e}
	9OS:1OPC	8.23 ± 0.12	11.62 ± 0.48^{b}	15.25 ± 0.28^{b}	24.73 ± 0.78^{b}	0.84 ± 0.01^{b}	0.23 ± 0.01^{c}	0.190 ± 0.01^{c}
	8OS:2OPC	8.16 ± 0.04	11.53 ± 0.50^{b}	15.84 ± 0.07^{b}	24.56 ± 0.58^{b}	0.76 ± 0.02^{c}	0.24 ± 0.03^{c}	0.147 ± 0.00^{de}
	7OS:3OPC	8.27 ± 0.18	12.04 ± 0.43^{b}	15.54 ± 1.94^{b}	28.12 ± 0.62^{a}	0.78 ± 0.02^{bc}	0.22 ± 0.00^{c}	0.141 ± 0.01^{de}
	Mean	**8.32** ^{a}	**12.57** ^{c}	**17.38** ^{c}	**24.23** ^{c}	**0.87** ^{c}	**0.259** ^{a}	**1.91** ^{b}
He-Trabzon	Control	8.93 ± 0.18^{a**}	16.29 ± 0.02$^{a\ **}$	26.42 ± 0.36$^{a\ **}$	36.52 ± 0.38$^{a\ **}$	1.05 ± 0.00$^{a\ **}$	0.24 ± 0.01$^{cd\ **}$	0.218 ± 0.01$^{b\ **}$
	9OS:1CHS	8.20 ± 0.07^{b}	13.25 ± 0.18^{a}	20.43 ± 0.05^{b}	22.85 ± 0.81^{b}	0.73 ± 0.01^{d}	0.25 ± 0.01^{bc}	0.250 ± 0.01^{b}
	8OS:2CHS	8.49 ± 0.27^{ab}	12.40 ± 0.18^{b}	19.69 ± 0.36^{c}	24.95 ± 0.90^{b}	0.73 ± 0.01^{d}	0.27 ± 0.01^{ab}	0.335 ± 0.02^{a}
	7OS:3CHS	8.81 ± 0.28^{b}	12.56 ± 0.35^{b}	20.90 ± 0.31^{b}	28.10 ± 0.92^{b}	0.83 ± 0.02^{cd}	0.27 ± 0.01^{a}	0.334 ± 0.00^{a}
	9OS:1OPC	8.52 ± 0.08^{ab}	11.38 ± 0.17^{c}	16.28 ± 0.13^{d}	34.00 ± 4.93^{a}	0.86 ± 0.10^{c}	0.22 ± 0.00^{d}	0.205 ± 0.00^{a}
	8OS:2OPC	8.63 ± 0.19^{ab}	11.62 ± 0.12^{c}	13.83 ± 0.18^{e}	35.15 ± 0.65^{a}	0.93 ± 0.03^{bc}	0.23 ± 0.00^{d}	0.195 ± 0.01^{a}
	7OS:3OPC	8.57 ± 0.15^{ab}	11.50 ± 0.11^{c}	11.17 ± 0.25^{f}	36.89 ± 0.09^{a}	0.98 ± 0.01^{ab}	0.22 ± 0.00^{d}	0.187 ± 0.01^{a}
	Mean	**8.59** ^{a}	**12.71** ^{c}	**18.39** ^{b}	**31.21** ^{b}	**0.87** ^{c}	**0.244** ^{b}	**2.46** ^{a}
He-İzmit	Control	8.69 ± 0.22^{ns}	20.24 ± 0.59$^{a\ **}$	22.27 ± 0.12$^{a\ **}$	43.00 ± 0.98$^{a\ **}$	1.16 ± 0.01$^{a\ **}$	0.24 ± 0.01$^{bc\ **}$	0.160 ± 0.00$^{a\ *}$
	9OS:1CHS	7.99 ± 0.38	12.61 ± 0.12^{bc}	17.20 ± 1.13^{cd}	32.77 ± 0.07^{bc}	0.89 ± 0.00^{c}	0.24 ± 0.00^{c}	0.125 ± 0.01^{a}
	8OS:2CHS	7.87 ± 0.42	12.95 ± 0.63^{b}	19.40 ± 0.32^{b}	28.84 ± 1.53^{c}	0.82 ± 0.04^{c}	0.27 ± 0.00^{ab}	0.122 ± 0.01^{a}
	7OS:3CHS	8.18 ± 0.20	12.87 ± 0.60^{b}	18.88 ± 0.66^{bc}	36.34 ± 2.98^{b}	1.00 ± 0.08^{b}	0.27 ± 0.01^{a}	0.111 ± 0.00^{a}
	9OS:1OPC	8.14 ± 0.42	11.59 ± 0.12^{c}	15.96 ± 0.24^{de}	31.96 ± 1.23^{c}	0.90 ± 0.02^{c}	0.26 ± 0.01^{ab}	0.141 ± 0.01^{a}
	8OS:2OPC	7.81 ± 0.50	12.15 ± 0.23^{bc}	14.32 ± 0.84^{e}	31.00 ± 1.08^{c}	0.85 ± 0.01^{c}	0.23 ± 0.02^{c}	0.148 ± 0.01^{a}
	7OS:3OPC	7.92 ± 0.19	12.12 ± 0.06^{bc}	17.82 ± 0.22^{bc}	29.51 ± 0.28^{c}	0.90 ± 0.02^{bc}	0.26 ± 0.00^{abc}	0.147 ± 0.01^{a}
	Mean	**8.08** ^{b}	**13.50** ^{b}	**17.98** ^{b}	**33.35** ^{a}	**0.93** ^{b}	**0.253** ^{a}	**1.36** ^{c}

n.s. – no significant; * – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$.Mean values in the same column within each isolate followed by the same letters are not significantly different by Tukey's tests.

growing medium and the isolate x growing medium interaction were significant for macro and micro element content of the fruitbody (Table 3). The ash percentage of the fruitbodies of He-Trabzon was affected by the growing medium ($P < 0.01$), whereas that of He-Ankara, He-Denizli and He-İzmit isolates was not affected ($P > 0.05$). The protein content of *H. erinaceus* isolates ranged between 11.38 and 20.24%, depending on the isolate evaluated and growing medium (Table 4). The protein content of *H. erinaceus* was reported to be 22.5% by Mau et al. (2001). The protein content of the *H. erinaceus* isolates grown on media supplemented with cotton seed and olive press cake was found to be lower than the values mentioned in the literature, while the protein content of the fruitbodies grown in the control medium was consistent with the literature. *H. erinaceus* isolates are a good source of many macro and micro elements, but the amount of elements they contain is varies depending on the substrates and isolates. The highest P and Mg levels were measured in the fruitbodies grown in the control medium, followed by 7OS:3CSH. The Fe, Zn, and Cu contents of *H. erinaceus* isolates grown on the control medium were higher than those grown in other media, whereas the Mn content of isolates grown on media prepared with OPC was higher. These results show that there was no direct relationship between the macro- and micro-element content of the growing media and the nutrient content of the fruitbodies. So the nature of the supplemental material affects the macro and micro element concentration of fruitbody as well as macro and micro element content of the growing media. Moreover, the fruitbodies of He-Ankara contained high amounts of protein and Fe, whereas those of He-Denizli were rich in Cu, those of He-Trabzon were rich in Mn and Cu, and those of He-İzmit were rich in Zn (Table 5).

Jo et al. (2013) and Manzi et al. (1999) found K to be the element with the highest levels in fruitbodies, while several previous studies on different types of mushroom species have reported both K and P as being the main elements (La Guardia et al., 2005; Oyetayo, 2005; Mattila et al., 2001; Wang et al., 2000). In the present study, K and P were found to be main elements in the *H. erinaceus* fruitbody, while Na was the third major element in the ash of the fruitbodies.

When the P and K contents of the growing media and fruitbodies are compared, the P and K levels of the fruitbodies were considerably higher than those of the growing media. Indeed, the P and K concentrations of the fruitbodies of *Hericium* isolates were 5.3_14.52 times and 9.98_18.47 times, higher respectively, than those of the growing media. Jo et al. (2013) also reported that the K content of the fruitbody is 3_18 times greater than that of the substrates. Conversely, the Ca values were significantly lower in the fruitbody, although Jo et al. (2013) found no evidence of Na and Ca accumulation in the fruitbodies. In addition, similar to the present results, they reported that the Mg content of the fruitbodies was lower than in the growing media. In this study, the levels of Fe and Mn were higher in the growing media than in the fruitbodies, while the Cu and Zn levels were higher in the fruitbodies. Metal binding peptides, also called phytochelatin, are situated in most eukaryotic cells and some prokaryotes and they form complexes with metals such as Zn, Cu, Ni (Clemens, 2006). Lee et al. (2009) reported that the high accumulation of Zn and Cu in fruitbodies could be explained by these phytocelatins. Oyetayo (2005) stated that variations in element composition of fruitbodies might be related to the

Table 5
Micro element composition of fruitbody of *Hericium erinaceus* isolates grown on different growing media.

İsolates	Substrates	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
He-Ankara	Control	87.37 ± 4.81$^{a\ **}$	11.15 ± 0.28$^{ab\ **}$	90.47 ± 1.98$^{a\ **}$	10.26 ± 0.50^{ns}
	9OS:1CHS	92.64 ± 2.48^{a}	10.19 ± 0.08^{b}	62.22 ± 0.65^{c}	9.38 ± 1.76
	8OS:2CHS	86.38 ± 1.19^{a}	13.59 ± 3.27^{ab}	74.00 ± 5.27^{b}	9.33 ± 0.67
	7OS:3CHS	69.28 ± 5.86^{b}	13.76 ± 0.01^{a}	76.85 ± 1.22^{b}	9.06 ± 0.03
	9OS:1OPC	60.74 ± 0.49^{b}	13.05 ± 0.11^{ab}	35.54 ± 0.97^{d}	9.28 ± 0.16
	8OS:2OPC	62.11 ± 2.47^{b}	13.54 ± 0.09^{ab}	39.77 ± 0.35^{d}	9.37 ± 0.38
	7OS:3OPC	63.54 ± 1.06^{b}	13.10 ± 0.16^{ab}	41.20 ± 0.81^{d}	9.77 ± 0.42
	Mean	**74.58** ^{a}	**12.63** ^{b}	**61.00** ^{b}	**9.49** ^{b}
He-Denizli	Control	88.72 ± 4.04$^{a\ **}$	10.75 ± 0.49$^{d\ **}$	61.96 ± 0.40$^{a\ **}$	10.65 ± 0.59^{ns}
	9OS:1CHS	64.04 ± 3.92^{d}	9.54 ± 0.43^{d}	39.32 ± 1.16^{d}	10.22 ± 0.68
	8OS:2CHS	68.68 ± 2.56^{c}	13.73 ± 0.24^{b}	48.26 ± 1.05^{b}	10.73 ± 0.17
	7OS:3CHS	82.62 ± 1.44^{b}	12.03 ± 0.40^{c}	64.21 ± 3.09^{a}	10.86 ± 0.06
	9OS:1OPC	33.49 ± 1.58^{g}	18.25 ± 0.71^{a}	42.88 ± 2.78^{cd}	10.40 ± 0.05
	8OS:2OPC	42.62 ± 0.64^{f}	13.36 ± 0.33^{b}	46.79 ± 1.01^{bc}	11.04 ± 0.28
	7OS:3OPC	46.93 ± 1.67^{e}	13.57 ± 0.28^{b}	47.48 ± 1.12^{bc}	10.29 ± 0.07
	Mean	**61.01** ^{c}	**13.03** ^{b}	**50.13** ^{c}	**10.60** ^{a}
He-Trabzon	Control	79.51 ± 2.83$^{a\ **}$	12.62 ± 0.16$^{e\ **}$	43.15 ± 2.08$^{d\ **}$	11.42 ± 1.12$^{a\ **}$
	9OS:1CHS	70.58 ± 0.98^{b}	13.32 ± 0.11^{d}	55.30 ± 1.22^{b}	9.05 ± 0.07^{b}
	8OS:2CHS	70.36 ± 1.38^{b}	13.23 ± 0.09^{de}	52.26 ± 1.96^{c}	10.39 ± 1.11^{ab}
	7OS:3CHS	62.70 ± 3.42^{d}	14.59 ± 0.11^{c}	53.44 ± 0.70^{c}	10.39 ± 0.72^{ab}
	9OS:1OPC	62.39 ± 1.55^{d}	17.02 ± 0.45^{a}	33.16 ± 1.01^{f}	11.45 ± 0.18^{a}
	8OS:2OPC	70.03 ± 0.18^{b}	15.57 ± 0.11^{b}	37.58 ± 0.98^{e}	10.84 ± 0.11^{ab}
	7OS:3OPC	69.92 ± 2.33^{bc}	15.29 ± 0.34^{b}	61.50 ± 2.50^{a}	10.98 ± 0.04^{a}
	Mean	**69.36** ^{b}	**14.52** ^{a}	**48.05** ^{c}	**10.65** ^{a}
He-İzmit	Control	74.97 ± 2.81$^{c\ **}$	4.70 ± 0.43$^{d\ **}$	87.10 ± 7.26$^{a\ **}$	10.88 ± 0.24^{ns}
	9OS:1CHS	80.60 ± 3.53^{b}	7.53 ± 1.30^{bc}	61.96 ± 1.49^{b}	10.44 ± 1.70
	8OS:2CHS	75.02 ± 4.60^{c}	9.63 ± 1.82^{ab}	66.76 ± 5.63^{b}	9.87 ± 0.63
	7OS:3CHS	94.40 ± 1.44^{a}	10.41 ± 0.02^{a}	95.38 ± 0.05^{a}	10.53 ± 0.51
	9OS:1OPC	54.74 ± 1.24^{e}	7.58 ± 0.06^{bc}	62.70 ± 1.08^{b}	9.16 ± 0.89
	8OS:2OPC	48.20 ± 1.14^{f}	6.25 ± 0.14^{cd}	65.34 ± 1.27^{b}	9.62 ± 1.30
	7OS:3OPC	56.33 ± 1.01^{d}	7.96 ± 0.92^{abc}	68.20 ± 9.31^{b}	10.56 ± 0.60
	Mean	**69.18** ^{b}	**7.72** ^{c}	**72.49** ^{a}	**10.15** ^{a}

n.s. – no significant; * – significant at P < 0.05; ** – significant at P < 0.01; *** – significant at P < 0.001. Mean values in the same column within each isolate followed by the same letters are not significantly different by Tukey's tests.

accumulation and availability of these elements in the growing media.

3.3. Effect of different growing media on antioxidant activity and the total phenol content of fruitbodies

Antioxidant activity was significantly influenced by the isolate ($P < 0.01$), the growing medium and the isolate x growing medium interaction ($P < 0.05$) (Table 3). Antioxidant activity in all the studied isolates ranged from 1.76 to 4.92 µmol TE/g fw. The highest antioxidant activity was measured in He-Izmit grown on the control medium, followed by those grown on media supplemented with OPC. Although Mau et al. (2002) reported that *H. erinaceus* fruitbodies exhibited low to moderate antioxidant activity as measured by the DETAB method, the present findings showed that, the methonolic extract obtained from the dry fruitbody of *H. erinaceus* was rich in phenolics and had potential ferric reducing antioxidant capacity.

The phenolic contents of *H. erinaceus* isolates determined in the study were higher that those obtained by Wong et al., (2009) (Table 6). Neither the antioxidant activity nor the total phenolic content of He-Ankara, He- Denizli and He- Trabzon isolates was affected by the growing media ($P > 0.05$). Both the antioxidant activity ($P < 0.05$) and total phenolic content ($P < 0.01$) varied significantly with the growing medium in He-Izmit. The addition of OPC to the oak sawdust substrate also had a noticeable effect on the phenolic content of the fruitbodies of He-İzmit which ranged between 0.49 mg GAE/g fw and 0.66 mg GAE/g fw. The highest phenolic content was determined in fruitbodies grown on 9OS:10OPC medium, followed by the 8OS:20OPC and 7OS:3OPC media. The literature includes different results and opinions about the effects of the growing media on fruitbody antioxidant activity and total phenolic content. Ruiz-Rodriguez et al. (2010) reported that different substrates did not affect fungal phenolic content because phenols were not absorbed by the fungi, although Singh et al. (2015) found that the antioxidant activity and phenolic content of *Pleurotus ostreatus* were influenced by different agro waste substrates. In addition, Koutrotsios et al. (2016) reported that *H. erinaceus* mushrooms grown on a substrate derived from olive pruning residues contained significantly higher total phenolics than mushrooms grown on beech sawdust. Conversely, Sanjust et al. (1991) found no accumulation of phenolics in fruitbodies grown on olive waste water. These conflicting results on the effect of the growing media on antioxidant activity and total phenolic content of mushrooms could be attributed to differences in the ability of mushroom species and isolates to utilise the growing medium.

Moreover, the strong positive correlation ($r = 0.936^{**}$) between the antioxidant activity determined by the FRAP assay and the total phenolic content of the fruitbodies of *H. erinaceus*, indicate the possible role of phenolic compounds in the antioxidant activity of *H. erinaceus*.

4. Conclusion

In conclusion, the CSH and OPC display potential as alternative additive materials and could become a cheap resource of *H. erinaceus*

Table 6
Antioxidant activity and total phenolic content of fruitbodies of *Hericium erinaceus* isolates grown on different growing media.

İsolates	Growing Substrates	Antioxidant activity micromol TE/g fw	Total phenolic content mg GAE/g fw
HE-Ankara	**OS:WB**	4.32 ± 0.290^{ns}	0.557 ± 0.006 ^{ns}
	9OS:1CSH	3.70 ± 0.04	0.543 ± 0.070
	8OS:2CHS	3.82 ± 0.09	0.570 ± 0.026
	7OS:3CHS	3.80 ± 0.09	0.587 ± 0.040
	9OS:1OPC	3.78 ± 0.14	0.553 ± 0.055
	8OS:2OPC	3.59 ± 0.18	0.523 ± 0.032
	7OS:3OPC	3.55 ± 0.24	0.507 ± 0.067
	Mean	**3.79^{ab}**	**0.548^{a}**
HE-Denizli	**OS:WB**	2.55 ± 0.45 ^{ns}	0.423 ± 0.099 ^{ns}
	9OS:1CSH	2.30 ± 0.52	0.337 ± 0.133
	8OS:2CHS	2.25 ± 0.83	0.390 ± 0.030
	7OS:3CHS	2.18 ± 0.18	0.320 ± 0.020
	9OS:1OPC	1.76 ± 0.37	0.353 ± 0.076
	8OS:2OPC	1.91 ± 0.39	0.340 ± 0.087
	7OS:3OPC	1.92 ± 0.28	0.360 ± 0.030
	Mean	**2.12^{c}**	**0.360^{b}**
HE-Trabzon	**OS:WB**	3.47 ± 0.87 ^{ns}	0.550 ± 0.075 ^{ns}
	9OS:1CSH	3.66 ± 0.23	0.507 ± 0.074
	8OS:2CHS	3.35 ± 0.37	0.543 ± 0.093
	7OS:3CHS	3.52 ± 0.40	0.490 ± 0.026
	9OS:1OPC	3.43 ± 0.39	0.560 ± 0.036
	8OS:2OPC	3.75 ± 0.17	0.583 ± 0.025
	7OS:3OPC	3.61 ± 0.37	0.577 ± 0.067
	Mean	**3.54^{b}**	**0.544^{a}**
HE-İzmit	**OS:WB**	4.92 ± 1.04 $^{a\ *}$	0.593 ± 0.098 $^{ab\ **}$
	9OS:1CSH	3.15 ± 0.83 ^{ab}	0.490 ± 0.046 ^{b}
	8OS:2CHS	2.85 ± 0.25^{b}	0.493 ± 0.012 ^{b}
	7OS:3CHS	3.59 ± 1.19^{ab}	0.520 ± 0.070 ^{ab}
	9OS:1OPC	4.48 ± 0.21^{ab}	0.663 ± 0.032 ^{a}
	8OS:2OPC	4.48 ± 0.31^{ab}	0.657 ± 0.067 ^{a}
	7OS:3OPC	4.28 ± 0.19 ^{ab}	0.650 ± 0.030 ^{ab}
	Mean	**3.97^{a}**	**0.580^{a}**

n.s. – no significant; * – significant at $P < 0.05$; ** – significant at $P < 0.01$; *** – significant at $P < 0.001$. Mean values in the same column within each isolate followed by the same letters are not significantly different by Tukey's tests.

cultivation. Moreover, as the growing medium affects the macro and micro element content of the fruitbodies of *H. erinaceus* isolates, the choice of growing medium may have an effect on the production of fruitbodies with desirable properties. The OPC could be considered a prospective substrate favouring the antioxidant activity and phenolic content of *H. erinaceus* isolates. Further research is needed to evaluate the effect of a wider range of materials on the mineral content and antioxidant properties of the fruitbodies.

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