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The effect of different fruiting temperatures on the yield and nutritional parameters of some wild and hybrid *Hericium* isolates

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ABSTRACT

Temperature has an important impact on the sporophore development of mushrooms. In particular, in some warm area or during summer season, temperatures above 20 °C may emerge as a limiting factor in *Hericium* spp. cultivation. The aim of this research was to assess the ability some isolates of *Hericium* spp. (*Hericium erinaceus*, *H. coralloides* and *H.americanum*) to produce fruitbodies at different temperatures (15, 20 and 25 °C). In addition, the effect of different fruiting temperatures on the yield parameters of *Hericium* spp. and the differences between some nutritional components in fruitbodies grown at different fruiting temperatures were also evaluated. Although some *H. erinaceus* isolates (He-Denizli, He-Erkel), *H. americanum* and *H. coralloides* formed pinheads and fruitbodies at 25 °C, other isolates tested in the study required lower temperatures (15 or 20 °C) for fruiting A noticeable decrease in the ash and protein content of all *Hericium* spp. isolates grown at 25 °C was verified. However, in general, macro-micro element content, antioxidant activity and phenolic content were not affected by fruiting temperatures. According to the results, some of the *Hericium* isolates tested in the study exhibited good potential for cultivation at 25 °C. Because of their ability to fruit with high yields under various temperature conditions, these isolates may be promising sources for breeding studies. Moreover, we have demonstrated that fruiting temperature is an important factor on yield parameters, protein and ash contents of *Hericium* spp.

1. Introduction

The *Hericium* spp. belongs to the family of *Hericiaceae* and are usually found naturally in autumn on standing or fallen trees, logs, stumps, and attached to dead branches (Boddy and Wald, 2003). Although there are different species within the *Hericium* genus including *Hericium americanum, H. abietis*, *H. laciniatum, H. coralloides*, *H. erinaceus* and *H. alpestre* (Park et al., 2004), most studies attempting to develop commercial cultivation methods have been focused on *H. erinaceus*.

H. erinaceus, also known as monkey head, lion's mane or pom pom mushroom, has been used for centuries in traditional Chinese medicine. This species was first cultivated in Japan in the 1960s (Chang et al., 1993) and nowadays is one of the important mushroom species commercially produced in the worldwide (Chang and Miles, 2004; Phan et al., 2014; Yamanaka, 2017).

H. erinaceus is notable because of its nutritional and culinary value as well as its medicinal properties. This mushroom is rich in protein (Cohen et al., 2014; Heleno et al., 2015; Atila et al., 2018), vitamin B12 (Teng et al., 2014), and minerals (Rodrigues et al., 2015; Heleno et al., 2015). It also possesses many medicinal properties through numerous bioactive compounds such as polysaccharides (Cheng et al., 2016; He et al., 2017), lectin (Li et al., 2010), hericirine (Li et al., 2014), hericenone (Shen et al., 2010), erinacol (Kenmoku et al., 2004) and erinacine (Shen et al., 2010; Wolters et al., 2015)., *H. coralloides* was first cultivated in 1984 (Chang and Miles, 2004) and although *H. coralloides* and *H. americanum* are not commercially important species today, many studies have demonstrated that these species also have medicinal properties such as antioxidant activity (Heleno et al., 2015; Kim et al., 2018; Atila, 2019a) and antibacterial (Julian et al., 2018), nerve growth factor promoter, anticancer (Wittstein et al., 2016) and anti-aging (Zhang et al., 2019) features.

Hericium spp. are efficient lignin degraders that can grow on a wide variety of agricultural and forest wastes. While *H. americanum, H. erinaceus* and *H. coralloides* also occur in eastern North America, there are

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two species, *H.erinaceus* and *H. coralloides*, that occur in parts of Europe and Asia (Grace and Mudge, 2015).Our previous research (Atila et al., 2017a, 2018) and other studies (Ko et al., 2005; Hassan, 2007; Figlas et al., 2007; Hu et al., 2008; Akdeniz, 2012; Koutrotsios et al., 2016) have shown that the growing medium has significant effects on the yield and nutritional value and chemical composition of *Hericium* spp. The process of cultivating mushrooms is affected by climatic factors as much as growing substrates. The temperature extremes that mushroom can tolerate depend on complex relationships, many of which are not well understood. Reports on the effects of different fruiting temperatures on yield of *Hericium* spp. are limited (Chiu, 1981; Oei, 1996), whereas there are no reports in the literature about the effects of fruiting temperatures on the nutritional content of *Hericium* spp. Under normal conditions, most mushrooms are not grown under a single temperature throughout their entire on all of their cultivation cycle. A temperature shock is needed for the mycelium to start the pinning stage (Chang and Miles, 2004). Thus, after the spawn running is completed at 25 °C, *H. erinaceus* is exposed to a lower temperature for pinning (Stamets, 1993). *H. erinaceus* strains are grown commercially at approximately 16−20 °C throughout the fruiting period. In some warm areas or during summer, the temperatures above 20 °C may appear to be a limiting factor in *Hericium* spp. cultivation in some warm area or during summer season. Therefore, in order to save cooling costs under these conditionsi it may be necessary to cultivate *Hericium* spp. strains that can form pinhead and fruitbodies at higher temperatures without the need for cooling.

Unfortunately, very few commercial strains of *H. erinaceus* are grown commercially in comparison with some other species such as oyster and shiitake mushrooms. In addition, it is quite difficult to purchase pure cultures of other *Hericium* species including *H. americanum* and *H. coralloides*. *H. erinaceus* production is common in countries of the Far East, although in other parts of the World, interest in *Hericium* spp. has increased greatly in recent years, especially due to its medicinal properties. Screening of local isolates from various habitats and geographic origins is important in order to popularize the production of this fungus in different regions. It is known that wild fungi or species of other organisms collected locally are better adapted to the conditions in their native area or region than those collected remotely (Uhart et al., 2008). Moreover, high-quality wild isolates that can grow under extreme conditions can become the source of new high-quality commercial isolates with high yields.

The aim of this study was to better understand the effect of different temperatures on the pinhead formation and development and yield parameters of *Hericium* spp. The study tested the abilities of eight *Hericium* isolates belonging to three different *Hericium* species of different origins to form pinheads and fruit bodies at different temperatures. The determination of the pinhead and fruitbody forming temperatures of isolates can provide a valuable resource for breeding programs aimed at producing commercial varieties that form fruit bodies at a high-temperature. In addition, although observations have often been made for *H. erinaceus* over a narrow temperature range, little is known about the effects of temperatures on the chemical composition of the mushrooms. For that reason, the effects of different temperatures on the yield parameters, protein, macro-microelement composition, total phenolic content and antioxidant activity of *Hericium* spp were also investigated in the study.

2. Materials and methods

2.1. Isolates

Hericium spp. isolates were collected from different areas of Turkey and from among those derived from the USA for a breeding project carried out by a mushroom spawn company (Agroma Co. Ltd., Denizli, Turkey) with the exception of the He-Erkel isolate, which was provided by Erkel Co. Ltd., (Izmir, Turkey). The isolates were recorded in Kırşehir Ahi Evran University culture collection with the isolation codes given in the Table 1. Pure cultures of *Hericium* spp. were stored on a malt extract agar (MEA) at 4 °C. Spawn production was carried out as previously defined by Atila (2017b).

2.2. Experimental design

Three experiments were conducted using two commercial and four wild isolates of *H. erinaceus,* one wild isolate of *H. coralloides* and one wild isolate of *H. americanum*. These isolates were exposed to three temperature regimes (15, 20 and 25 °C) to assess the effects of temperature on yield, productivity and nutritional content of the *Hericium* spp. The experiment was conducted in a randomized plot design at the Ege University, Department of Horticulture in Izmir (Turkey) in 2013–2014 years Analyses were carried out in the Research Unit Sustainable Use, Management and Reclamation of Soil and Water (GARSA) at the Technical University of Cartagena (Spain).

2.3. Preparation of growing substrate

Oak sawdust and wheat bran were mixed at ratios of 8:2 (w:w). The moisture of the growing substrate was adjusted to 70 % by adding tap water. Polypropylene bags (25 × 45 cm) were filled with prepared substrate (1 kg per bag) and autoclaved at 121 °C for 90 min. After cooling, the bags were inoculated using 3 % spawn (on a w:w wet weight basis) in a laminar flow chamber. Ten replicates were performed for each treatment.

The inoculated bags were placed to a spawn running room at 25 °C and 80–85 % relative humidity. When the spawn running period was completed, the bags were placed in three different mushroom cultivation rooms. The first set at 15 °C, second at 20 °C and the third at 25 °C. Light was provided 8 h of daily in all three rooms by cool white fluorescent bulbs. Ventilation was used to keep the CO_2 concentration below 1000 ppm and the relative humidity was set to 85–90 %. The harvests were made before the fruitbodies turned yellow and when the tassels were (around 5 mm in length). Total mushroom yield was obtained from two flushes.

The parameters evaluated to determine the effect of the temperatures of *Hericium* spp. cultivation included (i) earliness, described as the numbers of days from inoculation to the first primordia formation (ii) time to first harvest, expressed as the numbers of days from inoculation to the first harvest (iii) total yield, defined as the fresh weight of the mushrooms harvested (iv) biological efficiency (BE), calculated as the percentage ratio between total fresh mushroom weight and dry weight of the substrate and (v) average mushroom weight (AMW), described as the ratio between total yield and the total number of fruiting bodies per bag (Philippoussis et al., 2003).

2.4. Macro- and micro-element analysis

Mushroom samples were dried at 60 °C for 48 h in a drying oven and then ground and sieved in the laboratory using a 1 mm × 1 mm aperture mesh. Samples of 0.7 g were weighed into a porcelain crucible and then heated in a muffle furnace at 550 °C for 16 h.The ash samples were then digested with 0.6 mol L^{-1} nitric acid (HNO_3). The total nitrogen content

Table 1
Origins of *Hericium* spp. tested in the study.

Species	Strain Cod	Origin	Source
H. erinaceus	He- Ankara	Turkey	Wild
H. erinaceus	He-Denizli	Turkey	Wild
H. erinaceus	He-Erkel	Turkey	Commercial
H. erinaceus	He-Trabzon	Turkey	Wild
H. erinaceus	He-İzmit	Turkey	Wild
H. erinaceus	He-USA	USA	Commercial
H. americanum	HA	USA	Wild
H. coralloides	HC	Turkey	Wild

of samples was analized via the Duchaufour (1970) method. The crude protein content was calculated by using the adjusted conversion factor for mushrooms (N × 4.38) (Bano and Rajarathnam, 1988). Phosphorus (PO_4^{-3}) content was determined by spectrophotometer according to the MAPYA (1998) method. The K and Na were determined by emission using an AAS (AAnalyst 800) spectrometer system, while the Mg and Ca contents were analyzed by flame atomic absorption spectrometry. The micro elements (Fe, Zn, Cu and Mn) were determined by AAS spectrometry in accordance with the Madrid et al. (1996).

2.5. Total phenolic content and antioxidant activity

The Folin-Ciocaltaeu method (Swain and Hillis, 1959) was followed with some modifications to determine the total phenolic content of the methanolic extract of the *Hericium* spp. isolates. Five g of fresh fruitbody was homogenized in 25 mL of methanol using an Ika Ultra-Turrax homogenizer. The homogenate was stored in darkness for 14–16 h at 4 °C and then filtered through Whatman No. 4 filter paper (Thaipong et al., 2006). The absorbance of the supernatant was examined at 725 nm by a spectrophotometer (Bio 100, Varian, Australia). The total phenolic content in the extract was expressed as gallic acid equivalents per g of fresh weight (mg GAE g^{-1} fw).

The antioxidant activity in the *Hericium* spp. isolatess extracts was determined using the ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996). The absorbance values of the supernatant were read at 593 nm via a spectrophotometer (Bio 100, Varian, Australia). Trolox was used as a standard. The results were expressed as Trolox equivalents per g of fresh weight (μmol TE g^{-1} fw). The analysis were carried out with three replications.

2.6. Statistical analysis

The results of the experiments were presented as mean values and standard deviation (SD). The two-way analysis of variance (ANOVA) was used to determine statistical differences of the isolate and of the fruiting temperatures as well as their interaction on macro and micro element content, antioxidant activity, total phenolic content and on yield parameters. Statistical differences were then compared using Tukey's test at a significance level of 5%. These analyses were carried out using the SPSS 16.0 statistical package (SPPS, Inc)

3. Results and discussion

3.1. Effect of fruiting temperatures on earliness and yield parameters

The isolate, the fruiting temperatures and the isolate ^{x} fruiting temperatures interaction were significant for yield parameters (Table 2). In the study, He-Ankara, He-Trabzon, He-Izmit and HE-USA isolates did not form primordia and fruitbodies at 25 °C, whereas He-Denizli, He-Erkel, *H. americanum* and *H. coralloides* isolates formed primordia and fruitbodies at all three temperatures.

At 25 °C, *Hericium* isolates completed the spawn running period in 23–32 days. All wild *H. erinaceus* isolates required between 30.0 days and 39.8 days from inoculation to primordia initiation, whereas, at different temperatures the commercial isolatess completed the respective period in 26.9 days (He-Erkel-25 °C) and 38.5 days (He-USA-15 °C), ($P < 0.01$) (Table 3). The shortest time to primordia initiation lasted 32.3 and 30.6 days at 25 °C for *H. americanum* and *H. coralloides,* respectively. The data obtained revealed that both isolate and temperature could influence the time to pinhead formation. Moreover, previous studies have reported a large variation in the number of days taken for pinhead formation ranging from 24 to 45 days (Ko et al., 2005; Rodrigues et al., 2015; Koutrotsios et al., 2016; Atila, 2019b). The results obtained in this study fell within these limits. The isolate He-Erkel was significantly faster than the other isolates evaluated in the study, with the first harvest completed within 30 days from the inoculation at 25 and 20 °C. The other *H. erinaceus* isolates required between 33.6 days (He-Denizli- at 25 °C) and 48.5 days (He-Trabzon-15 °C), whereas for *H. coralloides* and *H. americanum* the respective period lasted 34.0 and 39.3 days at 25 °C. Figlas et al. (2007) reported that the first harvest of *H. erinaceus* cultivated on a growing medium containing sunflower seed hulls occured 35 days after inoculation and Atila (2019) confirmed that in different growing media, respective period varied between 29 and 36 days. However, *Hericium* spp. cultivated at 20 °C and 25 °C needed significantly shorter times to first harvest than when grown at 15 °C. This is commercially desirable since it reduces the duration of the production cycle.

Different species belonging to the same genus, and even isolates belonging to the same species, may need different temperatures for fruiting. Oei (1996) reported that fruitbody formation occurred at 20−28 °C in some *H. erinaceus* isolates, whereas others formed fruitbodies at 15−25 °C. It is recognised that in many species of basidiomycetes, the temperature must be reduced slightly for fruitbody formation. Chiu (1981) reported that temperature should be reduced to 20 °C for fruitbody formation in *H. erinaceus* cultivation, as in many mushroom species, otherwise fruitbody growth will slow down or even stop at 25 °C. Although all the *Hericium* isolates discussed in the experiment were cultivated in the same growing environment and under the same conditions (growing medium, light, CO_2, humidity) the reason that some isolates formed fruitbodies at 25 °C may have been due to the fact that these isolates do not require cooling shock for pin formation.

Yield and BEs for all wild isolates of *H. erinaceus* were ranged from 117.3 g kg^{-1} and 32.2 % BE (He-Denizli, 25 °C) to 65.6 g kg^{-1} and 18.0 % BE (He-Ankara, 15 °C), and these values were higher compared to those observed for the commercial strain He-USA (52.47 g kg^{-1} and 14.4 % BE at 20 °C and 53.51 g kg^{-1} and 14.7 % BEat 15 °C). The yield values obtained in the study are similar to previous findings (Hassan, 2007; Figlas et al., 2007; Akdeniz, 2012; Koutrotsios et al., 2016; Atila et al., 2018; Atila, 2019b), but lower than the results of Ko et al. (2005) and Hu et al. (2008). Interestingly, He-Denizli and He-Erkel isolatesgrown at 25 °C produced the same yield as when grown at 20 °C. Moreover, these values were higher than those of the same isolates grown at 15 °C (75.3 g kg^{-1} and 72.6 g kg^{-1}), which equates to a 55.9 and 52.5 % difference in yield due to the fruiting temperature. This can be advantageous in tropical regions and during hot seasons. For isolates of *H. erinaceus*, the results for yield, BEs and average mushroom weight were lower at 15 °C than at 20 °C, except for He-USA isolate whose yield at 15 °C, equaled its yield at 20 °C. At 20 °C and 25 °C, *H. americanum* presented the highest yield (128.6 g kg^{-1} and 121.1 g kg^{-1}) and BEs (35.32 % and 33.24 %), which were, however significantly different from the respective values

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* isolates grown on different fruiting temperatures.

		Days to primordia initiation	Days to first harvest	Yield	Biological efficiency	Average mushroom weight
Isolate	*F*	1608	1946	274.0	274.1	87.5
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature	*F*	2672	3709	585.4	585.5	242.1
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Isolate x Temperature	*F*	3120	3888	112.3	112.3	53.1
	P	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3
Effect of different fruiting temperatures on earliness, yield, BEs and average mushroom weight of *Hericium* spp.

Species	Isolates	Temperature(°C)	Earliness(days)	First harvest(days)	Yield(g kg^{-1})	BEs(%)	AMW(g)
		15	35.1 ± 0.7**^{a}	46.3 ± 0.8**^{a}	65.6 ± 4.9**^{b}	18.0 ± 1.3**^{b}	42.1 ± 13.3**^{a}
	He-Ankara	20	33.7 ± 0.5^{b}	40.7 ± 0.5 ^{b}	82.8 ± 12.0 ^{a}	22.7 ± 3.3 ^{a}	41.4 ± 6.0 ^{a}
		25	0.00 ± 0.0^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{b}
		15	32.4 ± 0.7**^{a}	40.1 ± 0.4**^{a}	75.3 ± 7.6**^{b}	20.7 ± 2.1**^{b}	37.6 ± 3.8**^{b}
	He-Denizli	20	30.6 ± 0.5 ^{b}	35.0 ± 0.6 ^{b}	113.0 ± 8.8 ^{a}	31.0 ± 2.4 ^{a}	56.5 ± 4.4 ^{a}
		25	30.0 ± 0.6 ^{b}	33.6 ± 0.8 ^{c}	117.3 ± 10.0 ^{a}	32.2 ± 2.7 ^{a}	58.3 ± 4.6 ^{a}
		15	29.3 ± 0.5**^{a}	35.7 ± 0.5**^{a}	72.6 ± 7.1**^{b}	19.9 ± 2.0**^{b}	36.3 ± 3.6**^{b}
	He-Erkel	20	27.2 ± 0.6 ^{b}	30.3 ± 0.6 ^{b}	107.6 ± 8.4 ^{a}	29.6 ± 2.3 ^{a}	53.8 ± 4.2 ^{a}
		25	26.9 ± 0.8 ^{b}	30.2 ± 0.7 ^{b}	110.8 ± 13.7 ^{a}	30.4 ± 3.8^{a}	56.0 ± 6.4 ^{a}
H. erinaceus		15	39.8 ± 0.6**^{a}	48.5 ± 0.5**^{a}	77.6 ± 7.4**^{b}	21.3 ± 2.0**^{b}	38.8 ± 3.7**^{b}
	He- Trabzon	20	36.5 ± 0.7 ^{b}	42.2 ± 0.9 ^{b}	103.1 ± 11.9 ^{a}	28.3 ± 3.3 ^{a}	64.9 ± 16.5 ^{a}
		25	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}
		15	33.8 ± 0.4**^{a}	39.4 ± 0.8**^{a}	83.1 ± 7.7**^{b}	22.8 ± 2.1**^{b}	41.6 ± 3.9**^{b}
	He-Izmit	20	32.0 ± 0.6 ^{b}	36.0 ± 0.5 ^{b}	108.8 ± 9.7 ^{a}	29.9 ± 2.7 ^{a}	63.9 ± 16.0 ^{a}
		25	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{c}
		15	38.5 ± 0.7**^{a}	48.3 ± 0.6**^{a}	53.5 ± 5.5**^{a}	14.7 ± 1.5**^{a}	26.8 ± 2.7**^{a}
	He-USA	20	37.9 ± 0.7 ^{a}	45.1 ± 0.5 ^{b}	52.5 ± 8.8 ^{a}	14.4 ± 2.4 ^{a}	26.2 ± 4.4 ^{a}
		25	0.00 ± 0.0 ^{b}	0.00 ± 0.0 ^{c}	0.00 ± 0.0 ^{b}	0.00 ± 0.0 ^{b}	0.00 ± 0.0 ^{b}
		15	36.0 ± 0.8**^{a}	44.9 ± 0.7**^{a}	83.1 ± 8.8**^{b}	22.8 ± 2.4**^{b}	41.6 ± 4.4**^{b}
H. americanum	HA	20	33.9 ± 0.5 ^{b}	40.4 ± 0.7 ^{b}	128.6 ± 8.6 ^{a}	35.3 ± 2.4 ^{a}	64.3 ± 4.3 ^{a}
		25	32.3 ± 0.5 ^{c}	39.3 ± 0.6 ^{c}	121.1 ± 8.9 ^{a}	33.2 ± 2.4 ^{a}	59.4 ± 5.7 ^{a}
		15	33.6 ± 0.8**^{a}	39.1 ± 0.9**^{a}	76.6 ± 11.5**^{b}	21.0 ± 3.2**^{b}	38.3 ± 5.7**^{b}
H. coralloides	HC	20	31.3 ± 0.6 ^{b}	34.8 ± 0.6 ^{b}	101.2 ± 12.0 ^{a}	27.8 ± 3.3 ^{a}	46.5 ± 8.6 ^{a}
		25	30.6 ± 0.5 ^{b}	34.0 ± 0.6 ^{b}	62.7 ± 6.7 ^{c}	17.2 ± 1.9 ^{c}	38.9 ± 8.7 ^{b}

ns. *. **. Nonsignificant. significant at $P<0.05$ or $P<0.01$. respectively. Mean values in the same row followed by the same letters are not significantly different by Tukey's test.

of most the *H. erinaceus* isolates and *H. coralloides*. The highest yield (101.2 g kg^{-1}) and BEs (27.8 %) were obtained in *H. coralloides* grown at 20 °C. Although *H. coralloides* formed fruitbodies at 25 °C, the yield was 38 % lower than when grown at 20 °C. Furthermore, 25 °C temperature in the growing room reduced mushroom development and, allowed the development of competitive microorganisms better adapted to high temperatures such as green mold.

In warm regions and summer season, the use of isolates that produce fruitbodies at high temperatures could help reduce energy costs in mushroom cultivation rooms.The fruitbody formation at high temperatures appeared to be promising in *H. americanum,* the isolated isolate of *H. erinaceus* from Denizli, and the commercial isolate provided from Erkel Co. Ltd. These isolates tolerated a wide range of temperatures (15–25 °C) for fruiting body development, with a low delay of fruiting, even though some diversity between the yields of the isolates at different temperatures could be observed. Moreover, the He-Denizli isolate was able to mature rapidly at 25 °C. Thus, He-Denizli may be a promising source for breeding studies of *H. erinaceus,* because of its ability to develop fruiting with high yields under various temperature conditions. However, standards for its commercialization should be researched.

High average mushroom weight is a quality parameter that is generally preferred by consumers. Earlier studies revealed that different substrates (Atila et al., 2017a, 2018; Atila 2019) affected the average mushroom weight of *Hericium* spp. However, according to results of the present study, this also seems to be affected by the fruiting temperatures. Fruitbodies grown at different temperatures clearly showed differences in averege mushroom weight in all the *Hericium* spp, except for He-Ankara and He-USA, that yielded the same fruitbody sizes when grown at 15 and 20 °C. On the contrary, in the other *H. erinaceus* isolates, *H. coralloides* and *H. americanum*, smaller fruitbodies were obtained at 15 °C than when grown at 20 and 25 °C. This may be attributed to the fact that a positive relation exist between average mushroom weight and high mushroom yield (Philippoussis et al., 2003) As regards the average mushroom weight of *H.erinaceus*, it also presented a wide range of values among the isolates evaluated; however, it is noteworthy that the wild isolates produced heavier fruitbodies (37.6 g–64.9 g) in comparison with the commercial isolates (26.2 g–56.0 g). *Hericium* spp. stains grown at 20 °C produced the biggest fruit bodies.

3.2. Effect of fruiting temperatures on the protein content, ash and nutritional composition of fruitbodies

The effect of the isolate, the temperature and their interaction on the protein and ash contents of the *Hericium* spp. was found to be statistically significant ($P < 0.01$) (Table 4). The ash content of the *Hericum* spp. growing at the three different fruiting temperatures varied between 4.9 % and 10.0 %, while protein contents varied between 12.9 % and 20.7 % (Table 5) The ash contents of *H. erinaceus* and *H. coralloides* were reported to be 6.91 % and 9.31 % by Rodrigues et al. (2015) and Heleno et al. (2015), respectively, whereas the protein contents of these mushroom species were determined as 18.8 % and 7.25 %, respectively, by the same authors On the other hand, Atila et al. (2017a) reported that the ash and protein content of *H. americanum* grown on different substrates ranged between 7.7 and 8.2 % and 6.0–11.6 %, respectively. In the present study, it was observed that the protein and ash contents of *H. erinaceus* and *H. americanum* grown at 25 °C were lower than the findings of the previous studies. This study also showed that the fruitbody ash content decreased when grown at 25 °C. The *Hericium* spp. grown at 20 °C gave the highest ash content. Similarly, in the *Hericium* spp, some variations in protein contents were encountered with the different temperatures, even within the same species, but the degree of variability was higher at 25 °C than at 15 °C and 20 °C. The protein accumulation of the fruitbodies was promoted by the lower fruiting temperature. Fruitbodies of He-Denizli, He-Erkel, *H. americanum* and *H. coralloides* grown at 20 °C accumulated about 36.5 %, 18.7 %, 23.7 % and 11.5 % more protein than the fruitbodies of the same isolates grown at 25 °C. Moreover, the protein content of the *Hericium* spp. grown at 20 °C was the same or slightly higher than in the fruitbodies grown on 15 °C, except for the He-Trabzon isolate.

The higher protein content of fruitbodies grown at 20 °C could be related to the optimal fruiting temperature. At the optimal fruiting temperature, cultivated mushrooms are able to utilize maximum N from their growing media. This allows cultivated mushrooms to grow optimally and synthesize protein. Differences on the protein content of the *Hericium* spp grown on different temperatures may have been affected by enzyme activity in the fungal metabolism. On the other hand, during fruting period of *Hericium* spp.,the warmer temperature (25 °C) might have given the isolate a shorter period than occurred at 25 °C. The

Table 4
F test of the two-way ANOVA and its statistical significance (P) for isolate, temperature and their interaction in macro and micro element content, antioxidant activity and total phenolic content of *Hericium* spp. fruitboy grown on different fruiting temperatures.

		Protein	Ash	P	K	Mg	Ca	Na	Mn	Cu	Fe	Zn	Total Phenol	Antioxidant Activity
Isolate	*F*	71.0	58.2	232.5	297.0	264.4	0.91	86.7	89.3	78.4	708.8	563.7	23.91	4.4
	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
Temperature	*F*	2850	2909	1460	2104	2783	4.6	311.2	463.1	597.6	4668	2182	280.9	109.1
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Isolate x Temperature	*F*	184.0	124.1	200.7	296.1	392.4	1.4	42.5	70.0	85.3	673.6	345.5	48.3	13.4
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.173	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 5
Effect of different fruiting temperatures on macro element composition of fruitbody of *Hericium* spp.

Species	Isolates	Temperature (°C)	Protein (%)	Ash (%)	P (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Ca (g kg^{-1})	Na (g kg^{-1})
H. erinaceus	He-Ankara	15	18.5 ± 0.1**	8.8 ± 0.2**^{a}	27.8 ± 2.0**^{a}	38.1 ± 0.6**^{a}	1.10 ± 0.03**^{a}	0.25 ± 0.01**^{a}	1.25 ± 0.04**^{a}
		20	19.7 ± 0.7	8.4 ± 0.3 ^{a}	29.4 ± 0.8 ^{a}	39.5 ± 1.7 ^{a}	1.09 ± 0.01 ^{a}	0.25 ± 0.01 ^{a}	1.27 ± 0.03 ^{a}
		25	0.0 ± 0.0	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
	He-Denizli	15	16.4 ± 0.0**^{a}	9.0 ± 0.1**^{a}	27.0 ± 1.1^{ns}	25.8 ± 0.5^{ns}	1.04 ± 0.02^{ns}	0.27 ± 0.01^{ns}	2.02 ± 0.08^{ns}
		20	15.9 ± 0.8 ^{a}	8.6 ± 0.2 ^{a}	26.9 ± 0.4	25.9 ± 0.4	1.08 ± 0.01	0.26 ± 0.01	1.95 ± 0.14
		25	10.4 ± 0.4 ^{b}	4.9 ± 0.3 ^{b}	26.5 ± 0.7	24.9 ± 1.1	1.07 ± 0.02	0.27 ± 0.01	1.90 ± 0.05
	He-Erkel	15	15.9 ± 1.3**^{a}	7.2 ± 0.1**^{b}	27.4 ± 0.6 ^{ns}	30.4 ± 0.7^{ns}	0.94 ± 0.07^{ns}	0.30 ± 0.01^{ns}	1.87 ± 0.05^{ns}
		20	17.0 ± 0.2 ^{a}	8.7 ± 0.1 ^{a}	27.2 ± 0.9	30.8 ± 0.2	0.94 ± 0.00	0.30 ± 0.04	1.79 ± 0.22
		25	12.9 ± 0.4 ^{b}	4.9 ± 0.2 ^{c}	27.2 ± 0.2	30.6 ± 0.5	0.96 ± 0.01	0.28 ± 0.01	1.82 ± 0.04
	He- Trabzon	15	16.9 ± 0.1**^{a}	8.1 ± 0.5**^{a}	26.2 ± 0.7**^{a}	34.2 ± 0.7**^{a}	1.05 ± 0.04**^{a}	0.26 ± 0.02**^{a}	1.86 ± 0.07**^{a}
		20	16.0 ± 0.0 ^{b}	8.8 ± 0.0 ^{a}	26.7 ± 0.7 ^{a}	35.1 ± 1.5 ^{a}	1.07 ± 0.02 ^{a}	0.27 ± 0.02 ^{a}	1.86 ± 0.26 ^{a}
		25	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{a}
	He-Izmit	15	19.4 ± 0.0**^{a}	8.6 ± 0.8**^{a}	23.8 ± 0.3**^{a}	43.0 ± 1.1**^{a}	1.08 ± 0.02**^{a}	0.28 ± 0.05**^{a}	1.51 ± 0.04**^{a}
		20	19.8 ± 0.5 ^{a}	8.6 ± 0.0 ^{a}	23.1 ± 0.8 ^{a}	43.3 ± 0.7 ^{a}	1.07 ± 0.02 ^{a}	0.24 ± 0.00 ^{a}	1.52 ± 0.05 ^{a}
		25	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
	He-USA	15	19.0 ± 0.1**^{b}	10.0 ± 0.3**^{a}	26.4 ± 0.7**^{a}	37.7 ± 0.8**^{a}	1.28 ± 0.03**^{a}	0.24 ± 0.00**^{a}	1.59 ± 0.04**^{a}
		20	20.7 ± 0.2 ^{a}	8.4 ± 0.2 ^{b}	27.2 ± 0.5 ^{a}	37.7 ± 2.0 ^{a}	1.32 ± 0.03 ^{a}	0.24 ± 0.01 ^{a}	1.53 ± 0.09 ^{a}
		25	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
H. americanum	HA	15	17.0 ± 0.0**^{a}	8.0 ± 0.1**^{a}	22.1 ± 0.7^{ns}	34.1 ± 1.3^{ns}	1.17 ± 0.02*^{a}	0.24 ± 0.01^{ns}	1.45 ± 0.07^{ns}
		20	16.3 ± 0.5 ^{a}	8.1 ± 0.2 ^{a}	21.6 ± 0.4	34.8 ± 0.9	1.13 ± 0.01 ^{ab}	0.24 ± 0.00	1.46 ± 0.05
		25	13.0 ± 1.3 ^{b}	5.7 ± 0.3 ^{b}	21.2 ± 1.1	34.5 ± 0.7	1.09 ± 0.01 ^{b}	0.23 ± 0.01	1.42 ± 0.26
H. coralloides	HC	15	16.2 ± 0.1**^{a}	6.7 ± 0.0**^{b}	28.7 ± 1.1^{ns}	44.0 ± 1.1^{ns}	0.99 ± 0.03^{ns}	0.22 ± 0.00*^{a}	1.36 ± 0.04^{ns}
		20	17.4 ± 0.1 ^{a}	8.5 ± 0.2 ^{a}	28.1 ± 0.6	44.4 ± 1.0	1.00 ± 0.02	0.21 ± 0.01 ^{ab}	1.34 ± 0.11
		25	14.4 ± 0.6 ^{b}	5.7 ± 0.1 ^{c}	28.2 ± 1.5	44.2 ± 1.7	0.98 ± 0.03	0.21 ± 0.00 ^{b}	1.35 ± 0.04

ns. *. **. Nonsignificant. significant at $P<0.05$ or $P<0.01$. respectively. Mean values in the same row followed by the same letters are not significantly different by Tukey's test.

longer fruiting period and lower yield at 15 °C may have caused an increase in protein deposition relative to nitrogen uptaken that led to higher protein content in the fruitbodies grown at 15 °C C. Moreover, because high temperature promotes microbial activation, it affects the rate of degradation of organic matter.

The macro element and micro element content of mushrooms was not affected by the fruiting temperatures (P > 0.05) (Tables 5 and 6). At different fruiting temperatures, the *Hericium* spp. showed (on a dry weight basis) 21.2–29.4 g kg^{-1} P, 25.8–44.4 g kg^{-1} K, 0.94–1.32 g kg^{-1} Mg, 0.21–0.31 g kg^{-1} Ca, 0.12–0.20 g kg^{-1}, Na, 58.7–87.5 mg kg^{-1} Fe, 7.1–11.8 mg kg^{-1} Mn, 40.3–91.4 mg kg^{-1} Zn and 9.4–10.2 mg kg^{-1} Cu. The macro and micro element content of the *Hericium* spp grown at different temperatures is comparable to the findings of previous studies (Akdeniz, 2012; Cohen et al., 2014; Heleno et al., 2015; Atila et al., 2017a, 2018). The protein and macro-micro element contents were significantly lower in *H. americanum* than in most of the other *Hericium* spp., although it exhibited a higher yield and BE (83.1 g kg^{-1}- 128.64 g kg^{-1} and 22.8 %–35.3 %, respectively).

3.3. Effect of fruiting temperatures on total phenolic content and antioxidant activity of fruitbodies

The effects of temperatures on total phenolic content and antioxidant activity of the *Hericium* spp. isolates are given in Table 6. The antioxidant activity of the *Hericium* spp. grown at different temperatures ranged from 2.68 to 4.86 μmol g^{-1} fw, whereas total phenolic content varied between 35.8 and 59.8 GAE g^{-1} fw. The literature reports various results for the effects of the growing media on *Hericium* spp. antioxidant activity and total phenolic content. (Wong et al., 2009; Abdullah et al., 2012; Koutrotsios et al., 2016). However, to date, no study has been carried out on the effect of fruiting temperatures on the total phenolic content and antioxidant activity of the fruitbodies.

As shown in the Table 6, the total phenolic content and antioxidant activity of the fruitbodies of He-Ankara were affected by the fruiting temperatures (P < 0.01), whereas those of the other *Hericium* isolates were not affected. The different effects of growth temperatures on antioxidant activity and total phenolic content of the He-Ankara isolate can be attributed to intra species genetic variability. The fruitbodies with the higher total phenolic content and antioxidant activity were obtained at 15 °C in He-Ankara isolate, while the yields for this isolate were lower than at 20 °C. Although the yield of He-Ankara grown at 15 °C was lower, the phenolic content andantioxidant activity of the isolate increased at this temperature. The difference in antioxidant activity in the He-Ankara isolate due to fruiting temperature was 50.2 %, which was much higher than the 22.6 % difference in phenolic content caused by fruiting temperatures. However, at low temperatures, the phenolic content and antioxidant activity of the He-Ankara isolate increased and, yield decreased. Therefore, growing this isolate at 20 °C would be a better choice in terms of commercial production because this would to moderate phenolic content and antioxidant activity and a higher yield.

Table 6
Effect of different fruiting temperatures on micro element composition, total phenolic content and antioxidant activity of fruitbody of *Hericium* spp.

Species	Isolates	Temperature(°C)	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Antioxidant activity micromol TE g^{-1} fw	Total phenolic content mg GAE g^{-1} fw
		15	86.6 ± 3.3 ^{**a}	9.8 ± 0.4 ^{**a}	91.4 ± 0.9^{**a}	9.6 ± 0.1^{**a}	4.67 ± 0.38^{**a}	0.60 ± 0.05 ^{a}
	He-Ankara	20	86.3 ± 0.8 ^{a}	9.5 ± 1.1 ^{a}	91.2 ± 0.8 ^{a}	9.5 ± 0.7 ^{a}	3.11 ± 0.24 ^{b}	0.49 ± 0.04 ^{b}
		25	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{c}	0.0 ± 0.0 ^{c}
		15	87.0 ± 0.4 ^{ns}	10.3 ± 0.7 ^{ns}	71.3 ± 0.9 ^{ns}	9.8 ± 0.3 ^{ns}	3.32 ± 0.46 ^{ns}	0.36 ± 0.03 ^{ns}
	He-Denizli	20	86.8 ± 1.7	10.0 ± 1.1	71.7 ± 1.4	9.4 ± 0.4	2.68 ± 0.23	0.38 ± 0.03
		25	87.2 ± 1.7	10.3 ± 0.9	71.5 ± 1.5	9.7 ± 0.5	2.95 ± 1.11	0.38 ± 0.03
		15	87.5 ± 0.9 ^{ns}	11.5 ± 0.4 ^{ns}	56.1 ± 1.7 ^{ns}	9.6 ± 0.2 ^{ns}	3.15 ± 0.20 ^{ns}	0.43 ± 0.03 ^{ns}
	He-Erkel	20	86.0 ± 1.2	11.4 ± 0.3	56.8 ± 0.5	9.6 ± 0.5	3.29 ± 0.38	0.46 ± 0.03
		25	87.0 ± 0.5	11.1 ± 0.6	56.7 ± 1.0	9.6 ± 0.9	3.15 ± 0.18	0.50 ± 0.03
H. erinaceus		15	84.5 ± 1.0^{**a}	11.4 ± 0.1^{**a}	50.2 ± 1.2^{**a}	10.1 ± 0.2^{**a}	4.86 ± 0.63 ^{**a}	0.51 ± 0.05 ^{**a}
	He- Trabzon	20	84.3 ± 3.3 ^{a}	11.4 ± 0.1 ^{a}	49.2 ± 0.2 ^{a}	10.2 ± 0.1 ^{a}	3.78 ± 0.74 ^{a}	0.58 ± 0.06 ^{a}
		25	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
		15	80.3 ± 0.7^{**a}	7.1 ± 0.4^{**a}	82.8 ± 2.2^{**a}	9.5 ± 1.0^{**a}	4.66 ± 0.37^{**a}	0.52 ± 0.08^{**a}
	He-Izmit	20	80.8 ± 1.3 ^{a}	7.3 ± 0.8 ^{a}	83.2 ± 2.7 ^{a}	10.0 ± 0.6 ^{a}	4.20 ± 0.03 ^{a}	0.54 ± 0.02 ^{a}
		25	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
		15	75.7 ± 0.6^{**a}	11.5 ± 0.5^{**a}	61.4 ± 1.0^{**a}	9.9 ± 0.3^{**a}	3.48 ± 0.13^{**a}	0.53 ± 0.01^{**a}
	He-USA	20	75.4 ± 0.7 ^{a}	11.8 ± 0.2 ^{a}	61.8 ± 6.3 ^{a}	9.9 ± 0.2 ^{a}	3.71 ± 0.46 ^{a}	0.54 ± 0.06 ^{a}
		25	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}	0.0 ± 0.0 ^{b}
		15	74.0 ± 1.4 ^{ns}	8.9 ± 1.0 ^{ns}	84.7 ± 0.3 ^{ns}	9.9 ± 0.3 ^{ns}	3.65 ± 0.08 ^{ns}	0.54 ± 0.02 ^{ns}
H. americanum	HA	20	75.1 ± 0.4	9.0 ± 0.2	84.2 ± 1.4	9.8 ± 0.3	3.02 ± 1.50	0.51 ± 0.04
		25	74.6 ± 0.7	9.2 ± 0.1	84.3 ± 1.7	9.8 ± 0.4	2.92 ± 0.07	0.54 ± 0.02
		15	57.7 ± 2.1^{ns}	8.4 ± 0.2 ^{ns}	41.2 ± 1.2 ^{ns}	9.6 ± 0.1 ^{ns}	3.53 ± 0.09 ^{**b}	0.46 ± 0.06 ^{ns}
H. coralloides	HC	20	58.7 ± 0.7	8.4 ± 0.4	40.3 ± 1.3	9.8 ± 0.1	4.29 ± 0.20 ^{a}	0.52 ± 0.00
		25	57.9 ± 1.7	8.3 ± 0.4	40.7 ± 1.6	9.7 ± 1.1	3.46 ± 0.14 ^{b}	0.50 ± 0.01

ns. *. **. Nonsignificant. significant at $P<0.05$ or $P<0.01$. respectively. Mean values in the same row followed by the same letters are not significantly different by Tukey's test.

4. Conclusion

According to the results, *Hericium* spp. and even the isolates belonging to the *H. erinaceus* species require different temperatures for fruiting. Unlike He-Ankara, He-Izmit, He-Trabzon and He-USA isolates that do not form pinhead and fruitbody at 25 °C, it is clear that He-Denizli and He-Erkel isolates were productive at 25 °C at least as much as at 20 °C and formed fruitbodies earlier at 25 °C compared with 15 °C and 20 °C. Moreover, *H. americanum* and *H. coralloides* also have a good potential for cultivation at 25 °C. Although these isolates develop faster and exhibited a high yield at 25 °C, this temperature could negatively affect the protein content of the fruitbody. In general, high temperature did not affect the macro and micro element content, total phenolic content or antioxidant capacity of the fruitbody. Taken together, these findings suggest that He-Denizli, a wild *H. erinaceus* isolate, could a favorable start for breeding programs aiming to produce new commercial strains that form fruitbodies at high temperature. Additional studies are needed in order to determine the response of *Hericium* spp. to higher temperatures and how to best use this information in practice.

CRediT authorship contribution statement

F. Atila: Formal analysis, Validation, Investigation, Writing - original draft, Visualization, Project administration. **Y. Tüzel:** Conceptualization, Methodology, Validation, Supervision, Writing - review & editing, Funding acquisition. **A. Pekşen:** Conceptualization, Methodology, Validation, Supervision, Writing - review & editing. **A. Faz Cano:** Resources, Methodology, Writing - review & editing. **J.A. Fernández:** Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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