Journal of Soil Science and Plant Nutrition, 2013, 13(1), 143-151

Nutritive value, flavonoid content and radical scavenging activity of the truffle (*Terfezia boudieri* Chatin)

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Abstract:

In this study, the amounts of dry matter, moisture, crude ash, protein, fat and organic matter in *T. boudieri* were determined to be 89.75, 10.25, 7.80, 20.13, 3.45 and 81.95% of the dry weight, respectively. The contents of mineral were: macronutrients (mg/g, dry wt) K 63.8, Ca 0.27 and Na 0.2; micronutrients (mg/kg, dry wt) Fe 1455.0, Zn 42.5, Mn 15.8 and Cu 30.1. Toxic elements such as Pb, Ni, Cd and Co concentrations were found to be under the detection limit of the used metod. The distribution of fatty acid was found to be 16.13% $C_{16:0}$, 0.98% $C_{16:1}$, 2.98% $C_{18:0}$, 4.91%$C_{18:1}$, 69.74% $C_{18:2}$, 4.53% $C_{18:3}$ and 0.72% $C_{22:0}$ of the wet weight. The amounts of myricetin, kaempferol, naringin, naringenin and resveratrol were determined to be 1.75, 0.25, 98.75, 0.25 and 2.25 µg/g of the dry weight, respectively. And also, free radical scavenging activity was understood to be 22.24 %.

Keywords: DPPH, edible mushroom, fatty acid, flavonoid, nutritive value, *T. boudieri*

1. Introduction

Mushrooms are valuable healthy foods, low in calories, fats, and essential fatty acids, and rich in vegetable proteins, vitamins and minerals. Although edible wild mushrooms are sold in higher prices in markets than that of cultivated mushrooms, consumers still prefer these because of their flavour and texture. These have become increasingly important in our diet due to their nutritional, pharmacological characteristics (Manzi *et al.*, 1999; Riberio *et al*., 2009; Akyüz and Kırbağ, 2010; Akyüz *et al.* 2012a) and popular because of its delicious taste.

Truffles are edible hypogeous fruit bodies produced by various genera of fungi belonging to the class *Ascomycetes.* The hypogeous ascocarps of these fungi are known as truffles. Among these, *Terfezia* and *Tuber* are classified as different taxa of the *Pezizales* (Bejerano *et al.,* 2004). Underground members of the *Pezizaceae* are well distributed around the globe. The genera *Tirmania* and *Terfezia* are monophyletic, and are morphological species which correspond to phylogenetic species. Species grouped under *Terfezia* clade are found in semi-arid habitats, and have ornamented and spherical spores. These species are adapted to different types of soils which they exploittogether with specific hosts.

These are not mycorrhizal with trees. Although other factors might have played a role, host specialization and edaphic tolerances might be the main reason for the speciesdiversity (Diez *et al.,* 2002). *Terfezia* and *Tirmania* spp. form mycorrhizae mainly on the roots of the *Cistaceae* family, and different species of the genus *Helianthemum* (Dexhemier *et al.*, 1985; Fortas *et al.*, 1992), but they have other symbionts as well. This type of mycorrhizal fungus ramifies through the soil, absorbing nitrogen and other minerals, which are transported back to the host plant (Ewaze and Al-Naama, 1989). These plants and their associated fungi may play a major role in the maintenance of Mediterranean shrublands and xerophytic grasslands, and help prevent erosion and desertification (Honrubia *et al*., 1992).

Pezizales are found in arid and semi-arid zones of Syria, Iraq,Iran, Lebanon, Bahrain, Jordan, Kuwait, Saudi Arabia, Tunisia, Egypt, Algeria, Morocco, Italy, France, Spain and in the Sahara and the Kalahari Deserts (Lawrynowicz *et al.*, 1997; Al-Rahmah *et al.*, 2001; Al-Ruqaie, 2002; Diez *et al.*, 2002; Mandeel and Al-Laith, 2007; Trappe *et al*., 2008). People in the certain continents are considered as the largest various truffle consumers, and the truffle commodity is regarded as a costly delicacy as stated by Al-Laith (2010). These are also found in Turkey, in simiar zones (Gücin and Dülger, 1997). *T. boudieri i*s seasonal and it is a socio-economically important mushroom in Turkey. It is edible and grown in certain wild regions of the country. It usually appears in the arid and semi-arid zones following the rainy season from March to May (Akyüz *et al*. 2012b).

Our knowledge on the physiology, biochemistry, plant-fungus relations of the hypogeous members of Pezizaceae are fragmentary. General studies on the composition and nutritional value of truffles have been carried out in some countries where they are known and appreciated. *T. boudieri* is especially appreciated for its culinary value because of its intense aroma . It is especially hard to understand the characteristics of their fatty acid and flavonoid content and their antioxidant activity. Therefore, the aim of this research is to extend our knowledge on the nutritional value of *T. boudieri*, and also to analyze their fatty acids, flavonoid content and antioxidant activity within Turkey.

2. Material and Methods

2.1. Sample collection

The mushroom sample used in this study was obtained from the previous work (Akyüz *et al.* 2012b). A sample of wild fresh *Terfezia boudieri* Chatin was collected from the vicinity of Kadıköy, Baskil-Elazığ, Turkey (38° 26’ 541’’ N, 38° 41’ 752’’ E, 700 m, 13.05.2007). These were collected from the area during spring (from March to May). We identifical the location of the truffles from crevices that appear on the surface of the soil above the truffles. The samples were cleaned without washing, cut into slices, dried at room temperature and then stored.

2.2. Proximate analysis

The proximate compositions of *T. boudieri* including moisture, dry matter, crude protein, crude fat, crude ash and organic matter, were determined according to AOAC (1990) methods. Total amount of nitrogen (N) was determined by the Kjeldahl method. Crude protein was calculated as N×6.25. Crude fat was determined by using the soxhlet extraction method both with a solvent and with a total ash incineration at 550°C. Organic matter was calculated as % dry matter - ash.

2.3. Element analysis

Each mushroom sample air-dried at room temparature was re-dried at 105°C overnight and crushed with a mortar and pestle. The digestion of mushroom samples was performed by using a mixture of HNO_3: H_2SO_4: H_2O_2 (10:1:1, 12 ml for 1 g sample) and by heating these at 100°C for about 10-15 min. After cooling, 50 ml deonized water was added and then all was filtered. In order to determine all the materials, all the used

glasswares were cleared with deonized water. While amounts of Fe, Zn, Mn, Cu, Cr, Cd, Co, Ni and Pb were determined by atomic absorption spectrometer (Perkin-Elmer, 370, The Perkin-Elmer Corporation Norwalk Connecticut, USA), amounts of K, Ca and Na were determined by atomic emission spectrometer (Eppendorf Geratebau, Netheler+HINZ GMBH Hamburg, GERMANY) (AOAC, 1990).

2.4. Fatty acid analysis

2 gof fresh mushroom was finely grounded in a mill for fatty acid analyses and then these were extracted with hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). The lipid extracts were centrifuged at 10.000 g for 5 minutes and filtered, and then the solvent was removed on a rotary evaporator at 40°C. The extracted lipids were stored under -25°C until further analysis. Fatty acids in the lipid extracts were converted into methyl esters by using 2% sulphuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted with n-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver. 3) which were linked with a glass GC 10 computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter), Permabound 25 (Macherey-Nagel, Germany) and by using nitrogen as a carrier gas (flow rate 0.8 ml/min). The temperatures of the column, detector and injection valve were 130-220, 240 and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures analyzed under the same conditions.

2.5. Flavonoid analysis and the chromatographic condition

2 g of dry mushroom was homogenized in 5 ml 80% methanol. Homogenates were centrifuged at 5000 rpm at +4ºC. After centrifugation, the supernatant was further concentrated by reduced-pressure rotary evaporation. Each extract was re-suspended in dimethyl sulphoxide (DMSO) to produce a stock solution. Chromatographic analysis was carried out using a PREVAIL C18 reversed-phase column (15×4.6mm, 5µm, USA); the mobile phase was achieved with methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (Zu *et al.*, 2006). This mobile phase was filtered through a 0.45 µm membrane filter (Millipore), then de-aerated ultrasonically prior to use. Catechin (CA), naringin (NA), rutin (RU), resveratrol (RES), myricetin (MYR), morin (MOR), naringenin (NAR), quercetin (QU) and kaempferol (KA) were quantified by DAD separation at 280 nm for CA and NA, 254 nm for RU, MYR, MOR and QU, 306 nm for RES, and 265 nm for KA. Flow rate and injection volume were 1.0 ml/min and l0 µL, respectively. The chromatographic peaks of the extracts were determined by comparing their retention time with the reference standards. Quantification was carried out by the integration of the peak by using the external standard method. All chromatographic operations were carried out at the temperature of 25°C.

2.6. Antioxidant assay by DPPH free radical scavenging activity

The free radical scavenging effect in extracts was assessed by the decoloration of a methanolic solution of DPPH according to the method of Liyana-Pathiranan and Shahidi (2005). A solution of 0.135 mM DPPH was prepared in methanol and 4.0 ml of this solution was mixed with 50, 100 and 250 µL of the extract in DMSO. The reacting mixture was left in a dark at room temperature for 30 mins. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Quercetin and resveratrol were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH free radical scavenging activity (%) = (Abs control – Abs sample) / (Abs control) × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract / standard.

Table 1. Nutrient contents of *T. boudieri* in Turkey (%, dry wt)

Nutrient Contents (%, dry wt)						Species	References
Dry Matter	**Moisture**	**Crude Ash**	**Crude Protein**	**Crude Fat**	**Organic Matter**		
89.75±0.07	**10.25±0.14**	**7.80±0.12**	**20.13±0.35**	**3.45±0.03**	**81.95±0.07**	***T. boudieri***	**◄In this study**
93.67-93.96	6.04-6.33	4.57-8.98	12.3-12.4	5.00-5.54	93.67-93.96	*T. boudieri*	Yildiz *et al.* (2006)
-	-	12.88	17.19	6.40	-	*T. claveryi*	Ahmed *et a*l. (1981)
-	-	4.6	19.6	2.8	-	*T. claveryi*	Sawaya *et al.* (1985)
		5.4	27.2	7.4		*T. nivea*	
-	-	5.8	17.6	-	-	*T. claveryi*	Kaisey *et al.* (1996)
		6.9	19.2			*T. hafizi*	
-	-	4.25	15.95	6.95	-	*T. claveryi*	Murcia *et al.* (2003)
		8.2	22.5	19.9		*P. juniperi*	
-	-	1.15	11.9	0.89	-	*T. claveryi*	Al-Ruqaie (2002)
		1.30	6.58	1.10		*T. nivea*	
-	-	-	12.82-17.19	-	-	*T. boudieri*	Ibrahim & Saeed
			16.30			*T. nivea*	(1994)
-	-	5.90	8.02	-	-	*T. claveryi*	Al-Naama *et al.*(1988)
		4.90	13.84			*T. nivea*	
		5.60	10.49			*T. pinoyi*	

Each value is expressed as mean ± SD of three replicate analyses (n=3).

Table 2. Mineral element concentration of *T. boudieri* in Turkey (dry wt)

Macro elements			Micro elements									
K (mg/g)	Ca (mg/g)	Na (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Co (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	**Species**	**References**
63.8±1.7	**0.27±0.02**	**207.5±5.6**	**1455.0±9.5**	**42.5±2.1**	**15.8±1.2**	**30.1±1.2**	-	-	-	-	***T. boudieri***	**◄ In this study**
9.96	0.68	290.00	170.00	130.00	22.00	83.00	-	-	-	-	*T. claveryi*	Ahmed et al. (1981)
10.30	1.00	1.11	0.33	0.03	0.01	0.03	<0.005	-	0.002	<0.001	*T. boudieri*	Yildiz et al. (2006)

Pb, Co, Ni and Cd: analysized, but not detected (the concentrations were under the detection limit of the metod used). The limits are 5-25, 1-5, 1-5 and 0.4-2 mg/g, respectively, Each value is expressed as mean ± SD of three replicate analyses (n=3)

2.7. Statistical analysis

Experimental values were given as means ± standard deviation (SD). Statistical significance wasdetermined by one-way variance analysis (ANOVA). Mean values were found with SPSS 13.0 (SPSS, Chicago, Illinois, USA). Each experiment was repeated three times and the results were reached by calculating the average value for three experiments.

3. Results and Discussion

3.1. Nutrient Composition

Dry matter and moisture content were 89.75% and 10.25%, respectively (Table 1). With respect to the dry matter and moisture contents,Yildiz *et al.*, (2006) obtained 93.67-93.96% and 6.04-6.33% in *T. boudieri,* respectively (Table 1). Therefore, the dry matter content was low, but moisture content was higher than the previously reported.

Ash content (7.80%) determined was lower than reported earlier by Ahmed *et al*. (1981), Murcia *et al*. (2003), Yildiz *et al*. (2006), and higher than previously reported by Sawaya *et al*. (1985), Al-Naama *et al*. (1988), Kaisey *et al*. (1996), Al-Ruqaie (2002), Murcia *et al*. (2003), and Yildiz *et al.* (2006) see Table 1.

The content of crude fat was found to be 3.45% in *T. boudieri* (Table 1). The crude fat contents reported earlier were 0.89-19.9% in different truffles such as *Terfezias* pp., *Tirmania* spp. and *Picoa juniperi* (Ahmed *et al.*, 1981; Sawaya *et al*., 1985; Al-Ruqaie, 2002; Murcia *et al.*, 2003; Yildiz *et al.*, 2006) see Table 1. These results were different from the results of present work as seen in Table 1.

The protein concentrations of the studied legume seeds show that these can contribute to the daily protein need of 23.6 g/100 g for adults as recommended by the National Research Council (1974). The crude protein content (20.13%) determined was lower than what was reported earlier by Sawaya *et al.*, (1985) and Murcia *et al*., (2003), but higher than what was previously reported by Ahmed *et al*. (1981), Al-Naama et al. (1998), Ibrahim and Saeed (1994), Kaisey *et al*. (1996), Al-Ruqaie (2002), Murcia *et al.*, (2003), Yildiz *et al*. (2006). This difference was probably due to the analysis of mushroom samples being obtained from different regions (Table 1).

3.2. Mineral element contents

As seen in Table 2, the macro element data were different from the the data obtained in the present study (Ahmed *et al.* 1981; Yildiz *et al*. 2006). The high quantity of potassium, magnesium and calcium together with the high quantity of sodium, plus the essential elements like iron, manganese, zinc and copper allow us to consider these as an excellent source of bioelements. As stated by previous researchers, mushrooms are recommended to be used in the preparation of diets for individuals who have low levels of these mineral elements (Manzi *et al*., 1999; Riberio *et al*., 2009; Akyüz and Kırbağ, 2010). Although the mineral elements form a small proportion of the total composition of total body weight although they do not contribute to the energy value of the food, these are of great physiological importance particularly to the body metabolism. Furthermore, the microelement contents of *T. boudieri* found in present study were changeable to other investigation (Ahmed *et al*. 1981; Yildiz *et al.* 2006) shown in Table 2. The amount of soil element is directly related to the mineral element levels of the mushroom. Normally, the changes in mushroom's element levels are closely based on the relationship between mycorrhizal plant and the soil. While mushroom mycelium provides the necessary minerals for the plant, it also gets the item sit needs from the plant.

Toxic metals such as lead, cobalt, nickel and cadmium were also tried to be analysed in this work, but concentrations were under the detection limit of the used metod (see Table 2).

Table 3. Fatty acids composition of *T. boudieri* in Turkey (%, wet wt)

Fatty acids (%)																		
$C_{10:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1\ n9}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1\ n9}$	$C_{18:2\ n6}$	$C_{18:3\ n6}$	$C_{20:0}$	$C_{20:2}$	$C_{21:0}$	$C_{21:0}$	$C_{22:0}$	$C_{22:1}$	$C_{24:0}$	**Species**	**References**
-	-	-	**16.13**	**0.98**	-	**2.98**	**4.91**	**69.74**	**4.53**	-	-	-	-	**0.72**	-	-	***T. boudieri***	**◄ In this study**
-	2.1	1.3	17.0	1.4	1.4	4.5	6.9	45.4	5.8	3.7	-	-	0.4	4.0	1.9	3.9	*T. claveryi*	Murcia et al. (2003)
	1.2	0.4	8.0	2.5	0.9	2.2	23.5	53.0	2.1	2.1			-	0.8	2.0	1.5	*P. juniperi*	
-	2.1	-	23.5	-	-	12.2	-	62.2	-	-	-	-	-	-	-	-	*T. claveryi*	Kaisey et al. (1996)
	0.7		24.0			14.8		60.5									*T. hafizi*	
10.37	-	-	23.7	1.295	-	3.990	10.37	44.35	2.120	-	1.635	1.02	-	-	-	-	*T. boudieri*	Yildiz et al. (2006)
-	2.6-6.3	-	11.7-18.9	-	-	3.3-6.2	3.8-23.1	47.9-70.5	0.9-2.4	-	-	-	-	-	-	-	*T. melanosporum*	Harki et al. (2006)

(C16:0) palmitic acid; (C16:1) palmitoleic acid; (C18:0) stearic acid; (C18:1) oleic acid;(C18:2) linoleic acid; (C18:3) linolenic acid; (C22:0) behenic acid

3.3. Fatty acid composition

The present study describes the presence of various fatty acids in *T. boudieri* see Table 3. The fatty acids compositions showed high quantities of linoleic acid (69.74% $C_{18:2}$), which was followed by palmitic acid ($C_{16:0}$), oleic ($C_{18:1}$) and linolenic acid ($C_{18:3}$) as seen in Table 3. It seems that the quantity of linoleic acid is higher than that reported previously, but other fatty acid values are different than those reported by other researchers (Kaisey *et al.*, 1996; Murcia *et al*., 2003; Harki *et al.*, 2006; Yildiz *et al.*, 2006) shown in Table 3. Large quantitative differences, probably due to the heterogeneity of the samples analysed, were reported by Harki *et al.* (2006). Unsaturated fatty acid predominated over saturated fatty acid in all the studied mushrooms, which is consistent with other studies (Riberio *et al.*, 2009). Oleic acid is known to help the frequency of cardiovascular diseases to decrease and known for its effectiveness in reducing cholesterol levels (Tomas *et al.*, 2001; Puiggros *et al.*, 2002; Pacheco *et al.*, 2008). Linoleic acid is an essential fatty acid which cannot be synthesized by the human organism, due to the lack of desaturase enzymes required for its production. Linolenic acid is an essential fatty acid which is able to produce the omega-3 fatty acids series in human bodies, including eicosapentaenoic acid and docosahexaenoic acid. The omega-3 and -6 fatty acids are biosynthetic precursors of eicosanoids, meaning that their intake concentrations will strongly affect eicosanoids production, and, therefore, the organism's metabolic functions (Voet and Voet, 2004). Besides these, various studies have show that these can also decrease the total amount of fat in blood (cholesterol), and reduce the risk of cardiovascular diseases. A deficient intake of essential fatty acids can cause many problems, such as dermatitis, imunosupression and cardiac disfunctions as stated by Burtis and Ashwood (1996).

3.4. Flavonoid contents and DPPH free radical scavenging activity

The amounts of myricetin, kaempferol, naringin, naringenin and resveratrol consisted 1.75, 0.25, 98.75, 0.25 and 2.25 µg/g of the dry weight, respectively. Naringin content (98.75 µg/g) was very high, whereas kaempferol and naringenin contents were very low in *T. boudieri* (see in Table 4). Furthermore; rutin, morin, quercetin and catechin were not detected in *T. boudieri* (see Table 4). For humans, several health beneficial properties of dietary flavonoids are recognized for their antioxidant and antiproliferative effects which may protect the body from various diseases, such as cancers, cardiovascular disease and inflammatory (Middleton *et al.* 2000; Nijveldt *et al.*

Table 4. Flavonoid contents (µg/g, dry wt) and free radical scavenging activity of *T. boudieri* in Turkey

Flavonoid contents (µg/g)									DPPH (%)		
Myricetin	Kaempferol	Naringin	Naringenin	Resveratrol	Rutin	Morin	Quercetin	Catechin	Concentration (50 µL)	Species	References
1.75	**0.25**	**98.75**	**0.25**	**2.25**	-	-	-	-	**22.24±1.91**	***T. boudieri***	**◄ In this study**
-	-	-	1.00	-	3.00	0.25	0.50	-	13.01±0.15	*P. sajor-caju*	
-	-	-	1.25	0.50	-	0.25	0.25	-	11.78±2.27	*P. ostreatus*	
-	-	-	-	0.75	0.50	64.50	-	-	14.58±0.86	*P. florida*	Akyüz et al. (2012a)
3.75	-	-	1.25	0.25	0.25	3.50	0.25	-	13.11±0.71	*P. eryngii var. eryngii*	
2.00	0.25	-	2.00	-	0.25	-	-	150.75	24.67±0.72	*P. eryngii var. ferulae*	
11.75	0.25	-	1.75	0.50	-	-	0.25	396.0	17.25±0.81	*A. bisporus*	

2001). Upto our knowledge, in the literature, there is no study on the flavonoid of studied mushroom species. When compared with different mushroom species, it has been concluded that the types and the amounts of the flavonoids change see Table 4. And also, free radical scavenging activity was 22.24% (Table 4). The inhibition percent of DPPH quenching of the dried truffles ranged between 24.5 and 69.2% with an average of 30.6% (Al-Laith, 2010). Furthermore, the observed high correlation between the various assays employed and phenolic contents is a strong indication that these phenolics (total, free and flavonoids) are among the main sources of antioxidant activity in desert truffles as stated by Al-Laith (2010). It seems that the radical scanvenging activity of *T. boudieri* might be different than what was expressed in an earlier published report (Akyüz *et al*. 2012b). Flavonoid contents and antioxidant activity of mushrooms depend on its relation with the strain/type, the ascocarp size, harvest time, host plant, and the ecocystems. Thus, it is expected that truffles originating from different geographical origins can show some variations. Furthermore, it is also expected that the antioxidant attributes of the truffles may be affected by the nature and by the extent of association with its host root associate *Helianthemum* spp. as stated by Al-Laith (2010). In conclusion, it has been determined that *T. boudieri* is rich from the point of view of unsaturated fatty acids such as linoleic, oleic and palmitic acid, and especially flavonoid such as myricetin, kaempferol, naringin, naringenin and resveratrol.

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