

## Physicochemical Characteristics, Phenolic Profile, Mineral and Carbohydrate Contents of Two Truffle Species

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### ABSTRACT

The aim of this study was an investigation on chemical composition including phenolic profile, mineral and carbohydrate content of the *Terfezia claveryi* (black truffle) and *Tirmania nivea* (white truffle). The identification of carbohydrates and individual phenolic compounds was performed by High Performance Liquid Chromatography (HPLC). Total protein was determined by Kjeldahl method. Our research showed that *Tirmania nivea* had higher contents of protein than *Terfezia claveryi*. Among studied carbohydrates, glucose was detected at higher levels in both truffles. The mineral analysis showed that potassium and iron concentrations were found at high levels compared with other minerals. Higher contents of the examined phenolic compounds were determined in extracts of *Terfezia claveryi* compared to those of *Tirmania nivea*. Overall, these results support further examination of biochemical characteristics and verification of nutritional value of both the truffles.

**Keyword:** Mineral composition, Phenolic compounds, Proximal composition, *Terfezia claveryi*, *Tirmania nivea*, Truffles.

### INTRODUCTION

Amongst the edible mushrooms, the nutritious seasonal truffles have a number of distinctive characteristics. They have a high economic value and are the world's most expensive mushrooms (Luard, 2006). Compared to the common everyday consumed mushrooms, truffles have different chemical characteristics making them easily distinguishable (Hall, 2007; Jamali and Banihashemi, 2013; Luard, 2006). They have generally no stalk, no gills, and their mycelium grows underground. Truffles do not have the soft and fragile feature, instead mature truffles

have a tendency to be compact and firm (Hall, 2007). They have delicious taste and there are some reports about their potential health benefits (Wang and Marcone, 2011). Wild and cultivated mushrooms are well known to contain various polyphenolic compounds which are accepted as antioxidants because of their ability to scavenge free radicals (Lillian and Ferreira, 2007). Especially, researches show that truffles have antimicrobial (Gouzi *et al.*, 2011; Janakat *et al.*, 2004), antioxidant (Al-Laith, 2010) and hepato-protective activity (Janakat and Nassar, 2010). The most studied edible truffle species include: *Terfezia* spp, *Tuber melanosporum*, *Tuber*

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*brumale*, *Tuber aestivum* and *Tuber indicum* which are all black truffles and the white truffles include *Tuber magnatum*, *Tuber borchii* and *Tirmania nivea*. Since *Terfezia* and *Tirmania* are mainly widespread to the arid and semi-arid parts of the Mediterranean, therefore these mycorrhizal fungi are also called desert truffles (Hall, 2007; Luard, 2006).

Several studies on truffles' chemical composition have shown that their protein and amino acids contents are higher than those of other edible mushrooms. Also they are rich in fiber, fatty acids, minerals, and carbohydrates. These substances may promote human health (Bokhary and Parvez, 1993; Kagan-Zur et al., 2013; Murcia et al., 2003). Chemical composition and nutritional quality of truffles growing in different countries have been reported. There is a general agreement among these studies that truffles, including *Tirmania nivea*, have a high content of protein, carbohydrate, and fiber (Ahmed et al., 1981; Sawaya et al., 1985). Research evidenced that fresh *T. aestivum* and *T. magnatum* are rich sources of proteins (11–12.9 and 20.5–24% in dry weight, respectively), and carbohydrates (5.65 and 2.23% in dry weight, respectively) (Saltarelli et al., 2008). Also, several minerals, such as potassium, phosphorus, iron and calcium, as well as sulfur containing amino acids and some fatty acids (linoleic, palmitic, oleic), were generally determined in truffles (Wang and Marcone, 2011).

Desert truffles are considered to be one of the oldest foodstuffs. In Iran, desert truffles, locally known as “donbalan” are seasonal and socio-economically important wild growing edible mushrooms. Among the various known edible desert truffles, only two species of the black color truffles belonging to the genus *Terfezia* and one species of the white color truffles belonging to the genus *Tirmania* are found in Iran (Jamali and Banihashemi, 2012). In Iran as well as in many Gulf countries, the truffles typically emerge in the deserts after the

rainy period between February and April (Al-ruqaie, 2006).

However, no studies have been conducted on the nutritive value, especially the phenolic acids, minerals and carbohydrates of local types of Iranian truffles. Therefore, the present study was done to assess the chemical components of two types of truffles in Iran.

## MATERIALS AND METHODS

### Samples

Wild black (*Terfezia clavaryi*.) and white (*Tirmania nivea*) truffles were collected at once in April 2016, from 3 sites (Zarqan, Dodej and Shahriar) of Fars Province in Iran. One kg of fully developed truffles from each species was collected from each site and totally 3 Kg equal to 67 truffles were collected for each species. The samples were cleaned with distilled water, air-dried between papers and immediately kept at –20°C until analysis. Some of the samples were sorted, cleaned and packed in polyethylene bags and stored at 4°C for physical analyses. Desired features were evaluated in the next 48 hours.

### Physical Properties

The length and diameter of the truffles were measured using a micrometer caliper. The volume was determined by water displacement.

### Proximate Analysis

The proximate compositions of *Terfezia clavaryi* and *Tirmania nivea* such as dry matter, crude protein and crude fat were revealed according to AOAC (Chemists and Cunniff, 1990) methods. Total amount of Nitrogen (N) was determined by the Kjeldahl method. Crude protein was calculated as  $N \times 4.38$ . Crude fat was

determined by the Soxhlet extraction method.

### Carbohydrates

Contents of sucrose, glucose, fructose and xylose were determined by HPLC method of Hasnaoui *et al.* (2011) (Hasnaoui *et al.*, 2011). An HPLC system (Agilent Technologies 1200 series, Germany) equipped with a pump system and Refractive Index Detector (RID) for carbohydrate analysis was used. Carbohydrates were extracted from samples (five grams) with maceration in 15 mL of ultrapure water at room temperature for 24 hours. The extracts were then centrifuged at 8,000×g for 15 minutes and the supernatants were collected. Each sample was filtered over 0.45 µm membrane filters and examined. The separation of carbohydrates was performed using a Zorbax carbohydrate 5 micron, 4.6×250 mm. All analyses were carried out at 30°C and a flow rate of 0.8 mL min<sup>-1</sup>. The neutral monosaccharide was eluted isocratically using 100 mM NaOH for 35 minutes.

### Minerals

Each mushroom sample was air-dried at room temperature and drying was finished at 105°C overnight, crushed with a mortar and pestle. Minerals were determined by AOAC method (Chemists and Cunniff, 1990). About 1 g of mushroom dry matter was weighed in a crucible and was ashed at

550°C. The ash was then dissolved in 5 mL of HCl (20%) and the solution was transferred to a 50 mL volumetric flask, the final volume was achieved with distilled water. For some minerals, the digestion of mushroom samples was performed using a mixture of HNO<sub>3</sub> (Nitric acid): H<sub>2</sub>SO<sub>4</sub> (Sulfuric acid): H<sub>2</sub>O<sub>2</sub> (Hydrogen peroxide) (10:1:1, 12 ml per 1 g sample) and by heating these at 100°C for about 10-15 minutes. After cooling, 50 ml of deionized water was added and then all was filtered. Analysis of the trace metals was carried out using an atomic absorption spectrophotometer.

A GBC Savanta AAΣ Double-beam atomic absorption/Flame emission spectrometer (Australia) equipped with a hollow cathode lamp as radiation source, a hydride generator model HG3000 and GF 5000 furnace and a graphite furnace tube and deuterium background correction was shown in (Table1).

A PAL 2000 auto sampler for liquid samples was installed on the GFAAS. Measurements were performed in triplicates. All reagents used were of analytical reagent grade. Stock standard solutions at concentration of 1,000 mg L<sup>-1</sup> for each of the determined elements were purchased all from Merck for certified Reference materials of Germany. Deionized water with resistivity of 0.06 µS cm<sup>-1</sup> was generated with milli-Q water purification system (millipor, USA). Working standard solution was prepared separately for each element. Limits Of Detection (LOD) and Limits Of Quantification (LOQ) were reported in (Table2).

**Table1.** Details of analytical procedures used for metal determination.

| Minerals | Wavelength (nm) | Slit width (nm), current (mA) | Minerals   | Wavelength (nm) | Slit width (nm), current (mA) |
|----------|-----------------|-------------------------------|------------|-----------------|-------------------------------|
| Arsenic  | 193.70          | 1.00, 8.00                    | Magnesium  | 285.20          | 0.50, 5.00                    |
| Cadmium  | 228.80          | 0.50, 3.00                    | Manganese  | 279.50          | 0.20, 5.00                    |
| Calcium  | 422.70          | 0.50, 5.00                    | Mercury    | 253.70          | 0.50, 3.00                    |
| Copper   | 324.70          | 0.50, 4.00                    | Phosphorus | 213.6           | 0.50, 10.00                   |
| Iron     | 248.30          | 0.20, 7.50                    | Potassium  | 404.40          | 0.20, 6.00                    |
| Lead     | 217.00          | 1.00, 5.50                    | Sodium     | 330.20          | 0.50, 5.00                    |
|          |                 |                               | Zinc       | 213.90          | 0.50, 5.00                    |

**Table 2.** Limits of detection / quantification for the individual elements.

| P<br>(ppm) | Na<br>(ppm) | Mg<br>(ppm) | K<br>(ppm) | Hg<br>(ppm) | Fe<br>(ppm) | Cu<br>(ppm) | Zn<br>(ppm) | Cd<br>(ppm) | Ca<br>(ppm) | Pb<br>(ppm) | As<br>(ppm) |                  |
|------------|-------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|------------------|
| 0.050      | 0.090       | 0.010       | 0.150      | 0.001       | 0.020       | 0.050       | 0.015       | 0.001       | 0.030       | 0.001       | 0.001       | LOD <sup>a</sup> |
| 0.160      | 0.300       | 0.030       | 0.500      | 0.003       | 0.070       | 0.150       | 0.060       | 0.003       | 0.100       | 0.003       | 0.003       | LOQ <sup>b</sup> |

<sup>a</sup> Limit of detection, the lowest analyte concentration that produces a response detectable above the noise level of the system. <sup>b</sup> Limit of quantification, the lowest level of analyte that can be accurately and precisely measured.

### Determination of Phenolic Compounds by HPLC Analysis

Extraction of plant material for the determination of phenolic compounds by HPLC was performed using a common method (Lister *et al.*, 1994) with some modifications: frozen samples were ground into powder and then 0.2 g were extracted with 3 mL of a mixture of methanol and acetic acid (85:15) and incubated in the dark for 24 hours. The extracts were then sonicated for 15 minutes and centrifuged at 10,000×g for 20 minutes, and the supernatants were collected. Supernatants were transferred to new tube and n-hexane was added at the same volume and strong vortexes. The bottom solution was collected using a syringe and then filtered through a 0.45 µm membrane filter prior to injection.

HPLC analysis was performed using a liquid chromatography (Agilent 1200 series), equipped with a Diode Array Detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an auto sampler and a thermostat column compartment, on an Agilent, Eclipse XDB-C18, 5 µm (ID), 4.6X150 mm (FT) column, at a flow-rate of 1 mL min<sup>-1</sup>. Solvent gradient was performed by changing the ratio of solvent A (methanol) to solvent B (formic acid 1%) as follows: initial 10% A; 0 minute, 25% A; 10 minutes, 60% A; 20 minutes, 70% A; 30 minutes, 70% A; 40 minutes. The total running time was 30 minutes and the column temperature was 30°C. The injected volume of samples and standards was 20 µL and it

was done automatically using auto sampler. The chromatographic peaks of the phenolic acids were checked by evaluating their retention times with that of their reference standards. All experiments were performed in triplicates. Limits of detection / quantification for the individual phenolics and the standard linear ranges are shown below (Table 3).

### Statistical Analysis

Statistical analyses were carried out by using Statistical Package for Social Science (SPSS) for Windows version 16.0. The results found in the present study were reported as mean values (obtained from the three replications)±Standard Deviation (SD). Kolmogorov–Smirnov test was used for evaluation of normality distribution. Variables were analyzed by Student's *t*-test or Mann–Whitney U-test when two groups were compared. *P*< 0.05 was considered significant.

## RESULTS AND DISCUSSION

### Physicochemical Property

Some physical and chemical properties of fresh *Terfezia claveryi* and *Tirmania nivea* are shown in Table 4. The mean length and diameter of *Tirmania nivea* were 5.5 and 3.5 cm, respectively, that was similar to *Terfezia claveryi* length and diameter (5.2 and 3.8 cm, respectively). Weight and volume of truffles were more different. The mean volume and weight of *Terfezia claveryi* were 78.5 cm<sup>3</sup> and 76.5 g that were more than

**Table 3.** Limits of detection / quantification for the compounds detected using HPLC.

| LOD (mg L <sup>-1</sup> ) | LOQ (mg L <sup>-1</sup> ) | Linear range (mg L <sup>-1</sup> ) |                            |
|---------------------------|---------------------------|------------------------------------|----------------------------|
| 0.08                      | 0.25                      | 0.25-1.25                          | Sinapic acid               |
| 0.14                      | 0.45                      | 0.45-3.60                          | Gallic acid                |
| 0.30                      | 1.00                      | 1.00-10.0                          | Catechin                   |
| 0.13                      | 0.43                      | 0.43-3.44                          | Caffeic acid               |
| 0.16                      | 0.53                      | 0.53-10.0                          | Chlorogenic acid           |
| 0.01                      | 0.03                      | 0.03-2.5                           | Rutin                      |
| 0.23                      | 0.76                      | 0.76-6.08                          | Quercetin                  |
| 0.07                      | 0.23                      | 0.23-1.84                          | <i>p</i> -Coumaric acid    |
| 0.31                      | 1.02                      | 1.02-8.16                          | Coumarin                   |
| 0.23                      | 0.77                      | 0.77-6.16                          | Carvacrol                  |
| 0.26                      | 0.85                      | 0.85-6.80                          | Vanilin                    |
| 0.28                      | 0.92                      | 0.92-4.60                          | <i>trans</i> -ferulic acid |
| 0.17                      | 0.55                      | 0.55-5.5                           | Hesperedin                 |
| 0.32                      | 1.06                      | 1.06-6.36                          | Ellagic acid               |
| 0.18                      | 0.59                      | 0.59-10.0                          | Eugenol                    |
| 0.14                      | 0.47                      | 0.47-3.29                          | Hesperetin                 |
| 0.15                      | 0.5                       | 0.50-5.00                          | Rosmarinic acid            |

**Table 4.** Some physical characteristics and proximate composition of *Tirmania nivea* and *Terfezia claveryi* truffles.

| Truffles names           | Weight (g) | Volume (mL) | Length (cm) | Diameter (cm) | Dry mater (%) | Fat (% in DW) | Protein (% in DW) |
|--------------------------|------------|-------------|-------------|---------------|---------------|---------------|-------------------|
| <i>Tirmania nivea</i>    | 48±3.5     | 58.5±5.3    | 5.5±0.5     | 3.5±0.2       | 81.0±3.5      | 8.25±1.3      | 4.33±1.4          |
| <i>Terfezia claveryi</i> | 76.5±5.2   | 78.5±4.8    | 5.2±0.3     | 3.8±0.2       | 85.0±5.2      | 8.83±0.8      | 3.35±0.9          |

those of *Tirmania nivea* 58.5±5.3 cm<sup>3</sup> and 48±3.5 g respectively.

Dry matter was 81 and 85%, respectively, in *Tirmania nivea* and *Terfezia claveryi* (Table 4). One study found 89% dry matter in *Terfezia boudieri* (Akyüz, 2013). Therefore, dry matter content was lower than those previously reported.

The mean content of crude fat was found to be 8.25 and 8.85% dry matter in *Tirmania nivea* and *Terfezia claveryi*, respectively (Table 4). The crude fat contents reported previously were 0.89-19.9% in different truffles for example *Terfezia* spp., *Tirmania* spp. and *Picoa juniperi* (Al-ruqaie, 2006; Murcia *et al.*, 2003). Another study reported 3.45% fat in *Terfezia boudieri* (Akyüz, 2013). Comparison of the compositional difference of three popular Saudi Arabian truffles including two black desert truffles (Gibaah and Kholeissi) and one white truffle (Zubaidi) showed that the truffles' fat

content ranged from 2.81 to 7.42% (Sawaya *et al.*, 1985).

The mean content of crude protein was found to be 4.33 and 3.35% in dry weight in *Tirmania nivea* and *Terfezia claveryi*, respectively (Table 4). The crude protein content determined in this study was lower than that reported previously (Murcia *et al.*, 2003; Sawaya *et al.*, 1985) and higher than another report (Al-ruqaie, 2006). One study showed that the nutritional composition of the desert truffles (*Terfezia claveryi*) was composed of 16% protein (% dry weight) (Bokhary and Parvez, 1993). Protein content of three different Iraqi truffle species (*Terfezia claveryi*, *Tirmania nivea*, and *Tirmania pinoyi*) ranged from 8.02 to 13.84% (Hussain and Al-Ruqaie, 1999). This variation was probably because of the analysis of mushroom samples being obtained from different areas.



### Carbohydrate

Carbohydrate compositions of truffles are given in Table 5. Among four carbohydrates that were determined, glucose content was highest in both species. However, content of fructose was very low and sucrose and xylose showed contents below the limits of quantification. *Tirmania nivea* and *Terfezia claveryi* contained nearly similar values of glucose. Sugar composition of *Tirmania nivea* and *Terfezia claveryi* was investigated for the first time in this study. Previous work on chemical composition of truffle records only show total carbohydrate contents in some species of *Terfezia* (Ahmed et al., 1981; Al Delaimy, 1977). The chemical analysis of *T. pfeilii* indicated the presence of fructose, glucose, sucrose and sorbitol. Previous research that studied wild mushrooms, reported sucrose (5.38%), fructose (0.02%) and mannitol (2.26%) in *Tuber aestivum* (Ackerman et al., 1975).

### Minerals

As shown in Table 6, among the minerals studied, potassium was the most abundant mineral (24,900 and 24,500 mg kg<sup>-1</sup> dry weight), followed by phosphorus (7,880 and 7,620 mg kg<sup>-1</sup> dry weight), calcium (4,150 and 3,840 mg kg<sup>-1</sup> dry weight), and magnesium (3,890 and 1,390 mg kg<sup>-1</sup> dry weight) in *Tirmania nivea* and *Terfezia claveryi* respectively. Our results concur more or less within the ranges reported by other researchers. One study showed that potassium content was higher than the level of other minerals in *T. olbiensis* and *T. claveryi* (Kıvrak, 2015). It was reported that this truffle contained Mg (1,017 and 998 mg kg<sup>-1</sup> dry weight), P (837 and 469 mg kg<sup>-1</sup> dry weight) and Ca (458 and 718 mg kg<sup>-1</sup> dry weight) respectively. We observed higher levels in both the analyzed species (Table 6). The mineral analysis showed that among the microelements of the two truffles, iron concentrations were found at the high levels

**Table 5.** Carbohydrate composition (mg 100g<sup>-1</sup> dry weight) of *Tirmania nivea* and *Terfezia claveryi* truffles.<sup>a</sup>

| Carbohydrates | Terfezia claveryi | Tirmania nivea | Retention time (Min) |
|---------------|-------------------|----------------|----------------------|
| Fructose      | 0.3               | 0.3            | 8.6                  |
| Glucose       | 7                 | 6.7            | 8.9                  |
| Sucrose       | ND                | ND             | 11.3                 |
| Xylose        | ND                | ND             | 7.4                  |

<sup>a</sup> Mean of carbohydrate composition after analysis in triplicates. The contents were not significantly different (P> 0.05).

**Table 6.** Content of minerals (mg kg<sup>-1</sup> dry weight) in *Tirmania nivea* and *Terfezia claveryi* truffles.

|                |                 | <i>Tirmania nivea</i> | <i>Terfezia claveryi</i> |
|----------------|-----------------|-----------------------|--------------------------|
| Major elements | Ca <sup>a</sup> | 4150±300              | 3840±310                 |
|                | K               | 24900±1230            | 24500±1420               |
|                | Mg <sup>a</sup> | 3890±520              | 1390±230                 |
|                | P               | 7880±270              | 7620±420                 |
| Trace elements | Mn              | 38.2±11               | 39.9±11                  |
|                | Cu <sup>a</sup> | 16.7±21               | 36.7±19                  |
|                | Fe <sup>a</sup> | 1206±101              | 1461±127                 |
|                | Zn <sup>a</sup> | 39.6±12               | 28.2±21                  |
|                | As <sup>a</sup> | 0.259±0.02            | 0.657±0.03               |
|                | Cd <sup>a</sup> | 1.030±0.04            | 0.155±0.01               |
|                | Hg <sup>a</sup> | 0.582±0.02            | 0.405±0.01               |
|                | Pb              | 0.252±0.03            | 0.252±0.02               |

<sup>a</sup> Mean values of three independent replicates were compared and significant differences were observed (P< 0.05).

compared to other evaluated trace elements. Iron content was found 1,206 and 1,461 mg kg<sup>-1</sup> dry weight in *Tirmania nivea* and *Terfezia claveryi*, respectively. The other elements in descending order were manganese, copper and zinc in *Terfezia claveryi* while in *Tirmania nivea* they were zinc, manganese and copper. Both the truffles contained similar levels of manganese however *Terfezia* had more copper. The level of iron reported in this study was significantly higher compared to earlier published reports (Demirbaş, 2001). It was reported that *Tuber magnatum* contained 68 mg g<sup>-1</sup> dry weight of iron (Segneanu *et al.*, 2012). Overall in the literature, reported iron values in mushrooms were 31.3-1190 mg kg<sup>-1</sup> dry weight (Sesli *et al.*, 2008), and 50.1-842 mg kg<sup>-1</sup> dry weight (Gençcelep *et al.*, 2009). It is known that adequate iron level in a diet is very important in order to decrease the incidence of anemia. Iron is the building stone of hemoglobin, the oxygen-carrying pigment of erythrocytes. Unfortunately, the information on bioavailability of iron from mushrooms has been lacking. It was reported that *Tuber magnatum* and *Tuber melanosporum* contained Zn (34 and 39 mg g<sup>-1</sup> dry weight) and Fe (68 and 523 mg g<sup>-1</sup> dry weight) respectively (Segneanu *et al.*, 2012). Among wild-grown edible mushroom species, the greatest levels of Zn were observed in *Tricholoma equestre* (173.8 mg kg<sup>-1</sup> dry weight) and *Lepista nuda* (121 mg kg<sup>-1</sup> dry weight) (Yamaç *et al.*, 2007a). The mineral content varied highly between the truffles. The variations could be due to agro-climatic changes.

As it was shown in Table 6, the order of detrimental element levels were Pb, As < Hg < Cd and Cd < Pb < Hg < As in *Tirmania nivea* and *Terfezia claveryi*, respectively. The levels of cadmium were measured as 1.03 and 0.155 mg kg<sup>-1</sup> dry weight in *Tirmania nivea* and *Terfezia claveryi*, respectively. Cadmium contents of mushroom samples in other researches have been shown to be in the ranges 0.10–0.71 (mg kg<sup>-1</sup> dry weight) (Mendil *et al.*, 2005)

and 0.26–3.24 (mg kg<sup>-1</sup> dry weight) (Yamaç *et al.*, 2007b), respectively.

Lead concentration of *Tirmania nivea* and *Terfezia claveryi* was 0.252 and 0.252 mg kg<sup>-1</sup> dry weight, respectively. Lead contents of mushroom samples in the literature have been reported to be in the ranges: 0.75–7.77 (Tüzen *et al.*, 1998), 0.40–2.80 (Svoboda *et al.*, 2000), 1.43–4.17 (Tüzen, 2003), and 0.80–2.70 mg kg<sup>-1</sup> dry weight (Turkekul *et al.*, 2004), therefore, the lead results of our search showed lower levels to those previously reported. The average amount of As and Hg present in our mushrooms was, in general, below the maximum permissible concentration. Literature reported that heavy metal concentrations in mushroom are significantly higher than those in other agricultural crops. This implies that mushrooms have a very effective mechanism that allows them readily to absorb some heavy metals from the ecosystem. Numerous wild edible mushroom species have potential to accumulate excessive concentrations of heavy metals for instance iron, lead, manganese, cadmium, chromium, copper, nickel, zinc, chromium, aluminum, and mercury (Svoboda *et al.*, 2000). Some environmental factors, for example pH and metal concentrations in soil, organic matter amount, and fungal factors, such as morphological part of fruiting body, species of mushroom, development stages, age of mycelium, and biochemical compositions influence metal accumulation in macrofungi (Garcia *et al.*, 1998).

### Phenolic Compounds

In order to gain more insight into the biochemical compounds, the individual phenolic compounds were determined using high performance liquid chromatography method. The detailed phenolic profiles are presented in Table 7. Phenolic compounds including gallic acid, catechin, chlorogenic acid, rutin, *p*-coumaric acid, hesperidin and eugenol, were determined in *Terfezia*

**Table 7.** Phenolic compounds (mg 100 g<sup>-1</sup> dry weight) of *Tirmania nivea* and *Terfezia claveryi* truffles.

| Phenolic compounds         | Retention time (min) | <i>Terfezia claveryi</i> | <i>Tirmania nivea</i> |
|----------------------------|----------------------|--------------------------|-----------------------|
| Sinapic acid               | 16.5                 | ND                       | ND                    |
| Gallic acid                | 3.3                  | 1.1                      | ND                    |
| Catechin                   | 8.7                  | 3.3                      | ND                    |
| Caffeic acid               | 11.6                 | ND                       | ND                    |
| Chlorogenic acid           | 10.5                 | 1.1                      | ND                    |
| Rutin                      | 12.6                 | 0.1                      | ND                    |
| Quercetin                  | 21.6                 | ND                       | ND                    |
| <i>p</i> -Coumaric acid    | 15.6                 | 0.3                      | ND                    |
| Coumarin                   | 17.4                 | ND                       | ND                    |
| Carvacerol                 | 28.4                 | ND                       | ND                    |
| Vanilin                    | 13.5                 | ND                       | ND                    |
| <i>Trans</i> -ferulic acid | 16.3                 | ND                       | ND                    |
| Hesperedin                 | 18.5                 | 0.59                     | ND                    |
| Ellagic acid               | 19.02                | ND                       | ND                    |
| Eugenol                    | 23.7                 | 5.1                      | 4.3                   |
| Hesperetin                 | 22.4                 | ND                       | ND                    |
| Rosmarinic acid            | 19.2                 | ND                       | 1.5                   |

*claveryi*. On the other hand, only eugenol and rosmarinic acid were determined in *Tirmania nivea*. Higher content of the examined phenolic compounds were determined in *Terfezia claveryi* rather than in *Tirmania nivea*. Compared to *Tirmania nivea*, higher values of eugenol (5.1 mg 100g<sup>-1</sup> dry weight) were observed in *Terfezia claveryi*. The profiles of phenolic compounds in edible mushrooms have been recognized before (Kim et al., 2008; Palacios et al., 2011). Diverse phenolics, namely gallic, homogentisic, protocatechuic, *p*-hydroxybenzoic, *o*- and *p*-coumaric acids and other phenolic derivatives, such as 3,4-dihydroxybenzaldehyde, were previously reported (Villares et al., 2012).

However, this comprehensive analysis of phenolic compounds in Iranian truffles could be a helpful basis for further approval of using it as food with health benefits. Because of antioxidant, anti-inflammatory, antimicrobial, chemo-preventive and anticancer properties of phenolic compound, the studied truffles are natural products that have promising biological activities (Halliwell and Gutteridge, 1999). Therefore, the verification of these compounds in the

examined *Terfezia claveryi* and *Tirmania nivea* extracts could contribute to their many potential applications.

## CONCLUSIONS

In this study, a detailed phenolic profile, minerals, and selected carbohydrates of Iranian truffle (*Terfezia claveryi* and *Tirmania nivea*) extracts were reported. Comparison of the carbohydrate composition of these two truffles showed that both had nearly the same amounts of reducing glucose. Sucrose, xylose and fructose showed very small amounts, sometimes less than limits of quantification. Regarding minerals, major and trace element contents were evaluated and compared in both *Terfezia claveryi* and *Tirmania nivea*. Ca and Mg contents of *Tirmania nivea* were significantly higher while P and K content were almost the same in both truffles. Trace elements evaluations revealed that except for Mn and Pb, six other trace element contents were significantly different in the two truffles species. Contents of Zn, Cd and Hg were significantly higher in *Tirmania nivea*



while Cu, Fe and As showed higher levels in *Terfezia claveryi*. Phenolic compound contents were also different in *Terfezia claveryi* and *Tirmania nivea*. From seventeen evaluated phenolic compounds, only eugenol and rosmarinic acid were detected in *Tirmania nivea* but only seven phenolic compounds were detected in *Terfezia claveryi*. However, the overall results found in this study revealed that *Terfezia claveryi* showed a higher level of phenolic compounds compared to *Tirmania nivea*. Compared to other elements, iron showed higher values in both truffles, which make them useful edible mushrooms. Further investigations of phytochemicals confirmation of nutraceutical use of both black and white truffles are suggested.

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### خصوصیات فیزیکوشیمیایی، محتوای فنولی، مواد معدنی و کربوهیدراتهای دو گونه قارچ

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#### چکیده

هدف از این مطالعه بررسی ترکیبات فنلی، مواد معدنی و محتوای کربوهیدراتی دو گونه قارچ سفید (*Tirmania nivea*) و قارچ سیاه (*Terfezia clavaryi*) بود. بررسی محتوای کربوهیدراتی و ترکیبات فنلی بوسیله کروماتوگرافی مایع با کارایی بالا (HPLC) انجام شد. پروتئین تام باروش کژلداال اندازه گیری گردید. نتایج نشان داد که *Tirmania nivea* میزان پروتئین بیشتری نسبت به *Terfezia clavaryi* دارد. از میان کربوهیدراتهای مطالعه شده در هر دو گونه قارچ، گلوکز بیشترین مقدار را داشت. بررسی مواد معدنی نشان داد که هر دو قارچ مورد مطالعه محتوای آهن و پتاسیم بالایی داشتند. بررسی میزان فنول نشان داد که گونه *Terfezia clavaryi* در مقایسه با گونه *Tirmania nivea* میزان محتوای فنلی بیشتری دارد. در مجموع نتایج بدست آمده نشان داد که هر دو گونه قارچ بررسی شده موارد مناسبی برای بررسی بیشتر خصوصیات بیوشیمیایی و مواد مغذی می باشند.