



## Analysis of nutrient and antinutrient content of the truffle (*Tirmania pinoyi*) from Morocco

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### **Abstract**

The wild truffle is a recognized nutrient-rich food considered to have a high nutritional value in many countries worldwide. However, information on its nutritive values and phytochemical composition is very limited. The objective of this paper is to determine the nutrient value and the content of the most common anti-nutrients in edible mushroom (*Tirmania pinoyi*) from the eastern region of Morocco using standard methods. The results revealed that the energetical value is about (651 kcal/kg, fresh mass), Moisture (81.5 g/100 g, fresh mass), total carbohydrate (64.74 g/100g, dry mass), Crude protein (26.96 g/100g, dry mass), Crude fat (3.01 g/100g, dry mass), Ash (5.28g/100g, dry mass), insoluble ash (0.0047g/100g, dry mass) and dietary fiber (13.3 g/100g, dry mass). Copper was the most abundant trace element with a concentration of 65.3 mg/Kg dry mass. Cyanide, oxalate, mercury and arsenic were absent. Some of the risk elements, namely cadmium and lead were significantly lower in mushroom compared to their toxic levels according to World Health Organization's safe limits. These results suggest that the truffles analyzed represent a significant source of nutrients and are safe for consumption. A significant presence of saponins and steroids was also shown and indicates potential therapeutic properties.

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### **Introduction**

Mushrooms are valuable healthy foods, low in calories, fats, and essential fatty acids, and rich in vegetable proteins, vitamins and minerals (Sunan and Marcone, 2011; Hamza and Neffati, 2014). Although edible wild mushrooms are sold with higher prices in markets than cultivated mushrooms (Nounsi *et al.*, 2014), they are becoming increasingly important in our diet thanks to their believed nutritional value and also to some supposed pharmacological effects in addition to their delicious taste (Kagan-Zur and Roth-Bejerano, 2008; Dib-Bellahouel and Fortas, 2011; Saha *et al.*, 2012; Akyüz, 2013; Dahham *et al.*, 2016).

Truffle is a term used to refer members of the genera *Tirmania* and *Terfezia* in the family Terfeziaceae, order Pezizales. They are edible fungi, mostly endemic to arid and semi-arid areas

of the North-Africa, Mediterranean Region, and Arabian Peninsula; some of them have been found in South Africa and China (Kagan-Zur and Roth-Bejerano, 2008; Dahham *et al.*, 2016). They are socio-economically important fungi and are widely consumed in North Africa (Morocco, Algeria, Tunisia and Egypt) and in the Middle East (Saudi Arabia, Kuwait, Iraq, Iran, Lebanon, Syria and Jordon). The desert truffle is not so strongly flavored when compared with the European truffles (Pegler, 2002; Dib-Bellahouel and Fortas, 2011; Gajos and Hilszczanska, 2013).

General studies on the composition and nutritional values of truffles have been carried out in some countries where they are known and appreciated. In morocco, *T. pinoyi*, a North African species, grows in the East of the country and constitutes an important food for the local population. In the present work, we aim to increase the knowledge about the

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nutritional properties (protein content, lipid and mineral composition) and anti-nutrient properties of Moroccan species.

## Materials and Methods

### Samples

*Tirmania pinoyi* samples were collected from eastern region of Morocco in January 2016. The mushrooms were transported to the laboratory and immediately thoroughly cleaned, peeled, sliced, carefully dried (at 40°C), pulverized into fine powder and stored at ambient temperature in a dry and dark place until analysis. The samples were deposited in the laboratory of mycology of Mohammed V university, faculty of sciences. All analyses were performed from these mushroom powders in triplicate as follows :

### Moisture

The moisture was determined on 3.0 g of fresh samples by oven-drying at 105°C to constant mass (Akyüz, 2013; Hamza et al., 2016). The samples were allowed to cool at room temperature in a desiccator before being reweighed. Moisture content was then calculated.

### Total ash and insoluble ash

The ash content was determined by combustion of 1.0 g of dry mass at 550°C for 8h (Rao and Xiang, 2009; Hamza et al., 2016) until a white ash of constant weight is obtained. The percentage of total ash was calculated (Rao and Xiang, 2009). The determination of acid-insoluble ash was performed by adding 10 ml of dilute hydrochloric acid to the ash obtained from the determination of the total ash. The crucible was heated on a water bath for 10 minutes and filtered with an ashless filter paper. The filter paper together with the residue was transferred to the original crucible, which was then dried and ignited to constant weight. The content of acid-insoluble ash was then calculated (Rao and Xiang, 2009)

### Element analysis

The digestion of 1 g samples was performed using a mixture of nitric acid ( $\text{HNO}_3$ ), Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and sulfuric acid ( $\text{H}_2\text{SO}_4$ ) (10:1:1) and heating at 100°C for about 10-15 min. After cooling, 50 ml of deionized water was added and then a filtration was performed. (Akyüz, 2013). Amounts of Zinc (Zn), Copper (Cu), Lead (Pb), Cadmium (Cd), Selenium (Se), Arsenic (As) and Mercury (Hg) were determined using an atomic absorption spectrometer (Shimadzu GFA 7000).

### Total protein

Total amount of nitrogen was determined by the Kjeldahl method using 5.0 g of dry sample. A factor of 6.25 was used for conversion from total nitrogen into crude protein (Akyüz, 2013).

### Crude fat

The crude fat content of the samples was determined using soxhlet extraction method. The solvent used was petroleum ether. 3.0 g of dried samples were weighed and secured in a soxhlet extraction thimble. At the end of extraction, the solvent was evaporated and the flask dried, cooled and reweighed. The percentage of crude lipid was calculated.

### Total carbohydrate and total energy

Total carbohydrate content of the samples was estimated by difference of mean values (Hamza et al., 2016).

$$\text{Carbohydrate} = \text{total solids} - (\text{protein} + \text{lipids} + \text{minerals}).$$

Total energy was calculated using the equation (Pavel, 2013).

$$\text{Kcal} = 4 \times (\text{g protein} + \text{g carbohydrates}) + 9 \times (\text{g lipids}).$$

### Dietary fiber

The Pectin was extracted from 10.0 g of dry sample according to the procedure of Lefsih et al. (2016). Cellulose was determined by treating 3.0 g of dry sample with an alkali solution (4% NaOH). The mixture was heated at reflux temperature for 2 hours. Then, the solid was filtered and washed using distilled water. This treatment was performed thrice. The acid hydrolysis treatment was conducted on the fibers after alkali treatment (Johar et al., 2012).

### Antinutrient content

The phytate, oxalate and total steroids were determined according to the procedure of Nwosu et al. (2011). Saponins and alkaloids were determined using the AOAC standard method (AOAC, 1990). Cyanide was estimated according to the procedure of Tivana et al. (2014).

### Statistical analysis

Each experiment was replicated three times for each parameter, from which we derived the mean values and standard deviation (SD).

## Results and Discussion

The proximate compositions of *T. pinoyi* in the fresh mass (FM) and dry mass (DM) are presented in Table 1 for nutrients composition, Table 2 for elements analysis, Table 3 for dietary fiber and Table 4 for antinutrients composition. The results of the nutritional value show that carbohydrates were the most abundant macronutrients followed by proteins. Ash and fat contents were low. The energetic contribution of *T. pinoyi* sample was 651.5 kcal/kg. For dietary fiber, pectin content is twice as large as cellulose. Copper was the most abundant trace element. Cadmium and lead were significantly lower. Cyanide, Oxalate, Mercury and Arsenic were not detected. The concentration of saponins is high, while the alkaloids and phytates contents were low.

The moisture content of fresh samples of *T. pinoyi* was 81.5% which is in accordance with the results obtained by other authors, ranging from 90% to 75% (Sawaya *et al.*, 1985; Hussain and Al-Ruqaie, 1999; Sunan and Marcone, 2011; Dundar *et al.*, 2012). This is an indication that truffle is highly perishable because the high moisture content promotes susceptibility to enzyme activity and microbial growth which accelerates spoilage. The dried truffles are indeed known for their hygroscopicity (Pavel, 2013).

The carbohydrate content (obtained by difference as explained in method) was 64.76% on dry weight basis. This result is lower compared to that of *T. pinoyi* (82.59%) found by Stojković *et al.* (2013), but it's in accordance with the results obtained by other authors, ranging from 21% to 60% (Hussain and Al-Ruqaie, 1999; Murcia *et al.*, 2003; Sunan and Marcone, 2011). Nevertheless, considerable differences in composition are evident not only among species but also within the same species in accordance with data from various laboratories. The differences may be due in part to the analytical procedure for determining the carbohydrate and secondly to the different stages of fruit body development. Carbohydrates constitute more than one-half of truffle dry matter. This group comprises various compounds: sugars (monosaccharides, their derivatives and oligosaccharides) and both reserve and construction polysaccharides (glycans) (Pavel, 2013).

The crude protein constitute 26.96% DM, This result is higher compared to that of *T. pinoyi* (8.06%) published by Stojković *et al* (2013), but comparable with mean contents of 19 and 29% DM reported by other authors (Sawaya *et al.*, 1985; Hussain and Al-Ruqaie, 1999; Liu *et al.*, 2010; Kruzselyi and Vetter, 2014). Some differences in the composition

Table 1. Different nutrients (in g/100g of DM, and energy level in kcal/kg FM) of *Tirmania pinoyi* (mean±SD)

Nutrients	Values
Moisture (FM)	81.5±1.7
Energy	651.5
Total carbohydrate	64.74±0.3
Crude protein	26.96±0.11
Crude fat	3.01±0.01
Ash	5.28±0.19
Insoluble Ash	0.0047±0.0002

are evident due partly to the conversion factor used by different authors (4.38 or 6.25), in addition to the variability between species. However, the protein levels may have been overestimated due to a high proportion of non-protein nitrogen, in particular in the chitin (Pavel, 2013).

The lipid content was 3.0% DM; this is similar to that described in Saudi truffles, where the lipid content was 4.2 to 7.2% DM (Sawaya *et al.*, 1985; Hussain and Al-Ruqaie, 1999). The nutritional contribution of truffle lipids is limited due to low total fat content and a low proportion of desirable n-3 fatty acids (Pavel, 2013). The low truffles dry matter and fat could be the main reason of low energy (around 651 kcal/kg FM). Thus, truffles are a low-energy nutrient. Moreover, some carbohydrates are only partially digestible or indigestible (e.g. mannitol and chitin) but are calculated in order to determine the energy value. This is the reason why these results are also certainly overestimated.

The data in Table 1, referring to the ash content of *T. pinoyi*, are in accordance with those described for different dry truffle varieties by Murcia *et al.* (2003) (*Terfezia claveryi* and *Picoa juniper*), Stojković *et al.* (2013) (*Tirmania pinoyi*) and Sawaya *et al.* (1985) (*Terfezia claveryi* and *Tirmania nivea*). Total ash and insoluble ash contents are important indices to illustrate the quality as well as purity of truffles. Total ash includes "physiological ash", which is derived from the truffle tissue itself, and "non-physiological ash", which is often from environmental contaminations such as sand and soil. Total ash content alone is not sufficient to reflect the quality of truffle, since the truffle materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the insoluble ash content is another index to illustrate the quality of materials (Rao and Xiang, 2009).

Table 2. Elements of *Tirmania pinoyi* in (mg/kg DM), mean $\pm$ SD

Elements	Values
Copper	65.3 $\pm$ 0.3
Zinc	38.1 $\pm$ 0.2
Selenium	35.3 $\pm$ 0.2
Lead	0.23 $\pm$ 0.05
Cadmium	0.31 $\pm$ 0.03
Arsenic	Not detected
Mercurey	Not detected

Table 3. Fiber content of *Tirmania pinoyi* (in g/100g DM), mean $\pm$ SD

Fibers	Value
Pectin	8.96 $\pm$ 0.07
Cellulose	4.34 $\pm$ 0.03

Accurate prediction of the total ash and acid-insoluble ash of *T. pinoyi* is less important. Therefore, the element analysis by atomic spectroscopy improves the analysis efficiency. Seven elements were determined from the inorganic constituents (Table 2). The microelements have low concentrations, as in the majority of truffles (Dundar et al., 2012; Kruzselyi and Vetter, 2014). Copper content is the most important (65.3 mg/kg DM), followed by Zinc (38.1 mg/kg DM) while Selenium content was found to be 35.3 mg/kg DM, making *T. pinoyi* seem to be a promising source of this essential element. Considering the poisonous (or known as poisonous) elements, the incidence of Arsenic and Mercury were under the detectable limits. Pb and Cd were present in low quantities (respectively 0.23 and 0.31 mg/kg DM) compared to other studies (Kruzselyi and Vetter, 2014). This shows that the normal use of *T. pinoyi* has no toxicological risk.

Table 3 shows the dietary fiber content, which are represented by pectin and cellulose (respectively 8.96 and 4.34 g/kg dry matter). These results are in accordance with those obtained by other authors (Murcia et al., 2003; Dundar et al., 2012) for *Terfezia claveryi* (7.02 g/kg), *Tirmania nivea* (13.02 g/kg) and *Picoa juniper* (13.04 g/kg). Dietary fibers have an important role in human nutrition by maintaining a healthy digestive system as they speed up the transit of bowel contents and protect from colon cancer, diverticular diseases and irritable bowel syndrome (Dundar et al., 2012). Recent studies indicated that dietary fibers have also a significant

Table 4. Antinutrients composition of *Tirmania pinoyi* (in g/100g, and Steroids in mg/100g of DM), mean $\pm$ SD

Antinutrients	Value
Saponin	12.38 $\pm$ 0.01
Phytate	0.581 $\pm$ 0.005
Stéroid	176.2 $\pm$ 1.9
Oxalate	Not Detected
Alcaloïd	Not Detected
Cyanide	Not Detected

role in the reduction of blood cholesterol levels in hiperlipidemic individuals and reduce the risk of developing diabetes (Al-Amiri et al., 2011).

The results obtained for the antinutrient composition (Table 4) revealed that the saponins content was relatively high (12.38% DM). Saponins are known for their industrial applications as foaming and surface active agents. They also have beneficial health effects (Tippel et al., 2016). The steroids content was 0.18% DM and the level of phytate was found to be 0.581% DM, which is low. The low level of phytate could be attributed to the presence of a phytase enzyme (Jain et al., 2016), which degrades phytate in truffle. The cyanides, oxalates and alkaloids were not found. To our knowledge no study has extended the data on the composition of anti-nutrients in truffles. The low level of phytate in *T. pinoyi* would therefore be nutritionally advantageous.

## Conclusion

On the whole, edible mushrooms (*T. pinoyi*) from the eastern region of Morocco analysed in this study were found to be a good source of protein, fibre and minerals with no toxicological risk. These results make them an excellent food that can support a healthy and well-balanced diet. Further detailed analysis of the truffle species for other nutrients, anti-nutrients and secondary metabolites with medicinal potential, should be undertaken.

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