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Environmental and chemical study of Iraqi truffles

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ABSTRACT
Iraqi truffle species were collected from different areas (Anbar District). The truffle species were studied after identification. They included three species were studied after identification. They included three species: the first is the Jebbeh truffle (*Terfezia claveryi*), which has a light brown color; the second is the Haraka truffle (*Terfezia boudieri*), which has a black-dark brown color; and the third is the cream truffle (*Terfezia nivea*), which has a white to cream color. Samples were taken from the soil surrounding the truffles during collection and from the three species to determine their physical and chemical properties. The biomass ratios Fungi: Bacteria level from 0.107g to 9.080g, According to the method of operation followed. This was done using a light microscope, the percentages of (fungi + bacteria) ranged between 0.39% and 54.78% and 0.25% and 45.05%, respectively; While these ratios varied, This proves that bacteria contribute more to the soil ecosystem than fungi, based on the ratio of fungal to bacterial content. The ratio of bacteria to fungi can be better assessed using the fungi: bacteria ratio, rather than biomass or production rate . However, discrepancies in estimates of microbial production persist due to their complex responses to soil revival activities, as well as the total number of fungi and bacteria associated with the truffle root zone. as well as the total number of fungi and bacteria associated with the truffle rhizosphere. The chemical composition of the soil was as shown, The results indicated that the highest pH was 7.61, the highest salt concentration was $CaCO_3$ 370.23 g.kg-1, K was the most abundant element at 366 mg.kg-1, and Cl- were the most abundant ions in the soil at a total of 0.76 Cmol.kg-1. The amino acids contained in the three species were characterized, and the kind and number of the amino acids were studied. The amino acid content of T. claveryi truffle was 0.193 p.p.m. aspartic acid and 193.434 p.p.m. glutamic acid, and the overall amino acid condensatration was 346.633 p.p.m. While the T. boudieri truffle contained a number of amino acids. around (14) amino acids, the values ranged from 0.011 p.p.m. for aspartic acid to 79.365 p.p.m. for glutamic, with a total amino acid concentration of 1132.497 ppm. Finally, the T. nivea truffle contained 0.295 ppm for isoleucine and 233.5556 p.p.m. for alanine, with a total amino acid concentration of 556.367 p.p.m. Soil property differet among the three truffle species, as did the amount of nutrients, and the number of bacteria and fungi varied among the truffle species.

KEYWORDS: Iraqi truffles, brittle solid, soil fungi and bacteria, Amino acid.

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دراسة بيئية وكيميائية للكمأة العراقية

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الملخص
تم جمع أنواع الكمأة العراقية من مناطق مختلفة (قضاء الأنبار). تمت دراسة أنواع الكمأة بعد تحديد هويتها. وشملت ثلاثة أنواع تمت دراستها بعد تحديد هويتها. وشملت ثلاثة أنواع: الأول هو كمأة الجبة **(Terfezia claveryi)** ذات اللون البني الفاتح؛ والثاني هو كمأة الحركة **(Terfezia boudieri)** ذات اللون البني الداكن الأسود؛ والثالث هو كمأة الكريمة **(Terfezia nivea)** ذات اللون الأبيض إلى الكريمي. تم أخذ عينات من التربة المحيطة بالكمأة أثناء الجمع ومن الأنواع الثلاثة لتحديد خصائصها الفيزيائية والكيميائية. نسب الكتلة الحيوية الفطريات: البكتيريا من 0.107 جم إلى 9.080 جم، وفقًا لطريقة التشغيل المتبعة. تم ذلك باستخدام مجهر ضوئي، وتراوحت نسب (الفطريات + البكتيريا) بين 0.39٪ و 54.78٪ و 0.25٪ و 45.05٪ على التوالي؛ في حين تباينت هذه النسب، وهذا يثبت أن البكتيريا تساهم في النظام البيئي للتربة أكثر من الفطريات، بناءً على نسبة محتوى الفطريات إلى البكتيريا. يمكن تقييم نسبة البكتيريا إلى الفطريات بشكل أفضل باستخدام نسبة الفطريات إلى البكتيريا، بدلاً من الكتلة الحيوية أو معدل الإنتاج. ومع ذلك، لا تزال التناقضات في تقديرات الإنتاج الميكروبي قائمة بسبب استجاباتها المعقدة لأنشطة إحياء التربة، وكذلك العدد الإجمالي للفطريات والبكتيريا المرتبطة بمنطقة جذور الكمأة. كان التركيب الكيميائي للتربة كما هو موضح، أشارت النتائج إلى أن أعلى درجة حموضة كانت 7.61، وكان أعلى تركيز للملح هو **$CaCO_3$ 370.23** جم.كجم-1، وكان البوتاسيوم هو العنصر الأكثر وفرة عند 366 ملجم.كجم-1، وكانت الكلوريد هي الأيونات الأكثر وفرة في التربة بإجمالي 0.76 مول.كجم-1. تم توصيف الأحماض الأمينية الموجودة في الأنواع الثلاثة، وتمت دراسة نوع وعدد الأحماض الأمينية. كان محتوى الأحماض الأمينية في الكمأة **T. claveryi 0.193** جزء في المليون من حمض الأسبارتيك و193.434 جزء في المليون من حمض الجلوتاميك، وكان التكثيف الكلي للأحماض الأمينية 346.633 جزء في المليون. بينما احتوت الكمأة **T. boudieri** على عدد من الأحماض الأمينية. حوالي (14) حمضًا أمينيًا، وتراوحت القيم من 0.011 جزء في المليون. ارتفع تركيز حمض

الأســـبارتيك إلى 79.365 جزء في المليون لحمض الجلوتاميك، مع تركيز إجمالي للأحماض الأمينية قدره 1132.497 جزء في المليون. وأخيرًا، احتوى فطر الكمأة **T. nivea** على 0.295 جزء في المليون من الأيزولوســـين و233.5556 جزء في المليون من الألانين، مع تركيز إجمالي للأحماض الأمينية قدره 556.367 جزء في المليون. اختلفت خصـــائص التربة بين أنواع الكمأة الثلاثة، وكذلك كمية العناصر الغذائية، وتفاوتت أعداد البكتيريا والفطريات بين أنواع الكمأة.

الكلمات المفتاحية: الكمأة العراقية، المواد الصلبة الهشة، فطريات وبكتيريا التربة، الأحماض الأمينية..

INTRODUCTION

Truffles, large, underground ascomycetes, have been considered a delicacy for centuries and hold a prominent place in the culinary world. Their parts, as , anandamide tuberoside, polysaccharides, ergosterol and phenolics, along with their antioxidant, anti-inflammatory, antitumor, antimicrobial, immunomodulatory, and aphrodisiac benefits, have attracted the attention of international consumers. However, due to the significant gap between supply and demand, some species are expensive, limiting their availability and restricting their consumption to a select few. Nutritious food has become increasingly important, as a balanced, unprocessed diet plays a vital role in the prevention of several inflammatory diseases (Anand et al.,2015;Balestrini,et al.,2000). Truffles are edible subterranean fungi (growing 10–30 cm underground) (Taschen,et al.,2022). Their nutritional and gustatory value are very high. It is highly valued by chefs (haute cuisine), as evidenced by its nicknames "subterranean gold" and "kitchen diamond" (Bradai,et al., 2015). However, its consumers are few. Its seasonal production under specific conditions makes it expensive and unavailable to the entire world population. The global truffle harvest amounts to Several hundred tons are produced, but this amount is small compared to the enormous demand. Due to the significant gap between market supply and demand, the cost of obtaining one kilogram of truffles is very high. Desert truffles are of nutritional importance. They also belong to the subterranean ascomycetes, which are symbiotically associated with certain vascular plants. They are found in desert areas after each heavy rainy season (Ferdman et al., 2005). Tubers can be used as an excellent food, including black truffles, which are distinguished by their distinctive taste and unique aroma. black truffles are among the rarest and most valuable foods in the world (Hull et al., 2004). Therefore, efforts are increasing to cultivate it and study its nutritional and water requirements. Terfezia claveryi is one of the most widespread fungi and grows in gypsum clay soils. In calcareous and sandy clay soils, numerous desert truffle species are found in the desert regions of Iraq and some Middle Eastern countries (Gao JinMing et al., 2001). These include T. claveryi, known locally as jabba (dark brown with a thick, smooth skin), T. boudieri, known as harqa (black with a thick, smooth skin), and Termania nivea, known as zubaidi (white with a cracked skin) (Mandeel,et ai., 2007). Truffles are the fruit of certain fungi of the Ascomycetes family. Their mycelium forms mycorrhizae with the rhizome of organism lives plant species. Their increase depends on soil spiecalest (soil kind and climatic enverumint) and the truffle typs (Pacioni, et al., 2015). Several conditional parts, such as rainfall intensity and timing, soil type, and climate change, alter the chemical installation of the same truffle type from unlike zone (Ruoxy,et al., 1999). Even the steward plant influences truffle growth (Zhang LiFang,et al., 2005). show that two known fungal genera (Trevisia and Termania) contain numerous amino acids. This research aims to compare the three desert truffle species common in Iraq in terms of the amino acids present in each species and their amounts, In addition to the microorganisms in the soil surrounding the species under study. .

MATERIAL AND METHOD

Detection of Chemicals in Soil

The methods were used in USDA Guide No. 60 (1954) for Soil Analysis (pH, electrical conductivity, calcium carbonate, calcium sulfate, sodium, potassium, calcium, magnesium, chloride, hydrocarbons, and sulfate**.**

Detection of Fungal and Bacterial Content:

Soil organic matter was determined by detecting fungi and bacteria, using the Wookie and Black method as described by Jackson (1958). Available nitrogen was measured according to Black (1965a), and available phosphorus according to Olsen and Somers in Page et al. (1982**.(**

Sample Preparation:

The truffles were collected, washed, and cut into small pieces. Each piece was then placed on aluminum foil to dry in a preheated oven at 50°C for 48 hours. The dried truffles were ground into a powder, weighed, and stored in a sealed plastic bag at room temperature**.**

Sample (A) Extraction:

The sample was extracted using deionized water at 250°C and stirred for 6 hours. After centrifugation, the sediment and supernatant were collected after filtration and then freeze-dried in 6, 12, and 18-liter containers. The freeze-dried solution yielded deionized aqueous extracts**.**

Sample (B) Extraction :

An alcoholic extraction was performed using a solution of (80:20 ethanol :water) at room temperature for 7 days. The extract was then filtered and dried under vacuum using an evaporator, yielding 0.0 mM ethanol extracts. The absorbance was measured using UV-Vis spectroscopy at a wavelength of 517 nm**.**

Amino Acid Approximation of amino acid*:-*

The amino acids were determined using chromatography. (Dilution 1, Mobile Phase: Buffer Solution: Mixture, Detection: Ex = 340 nm, Em = 450 nm, Flow Rate: 2 mL/min). Saul and Wunsch Needleman (1970).

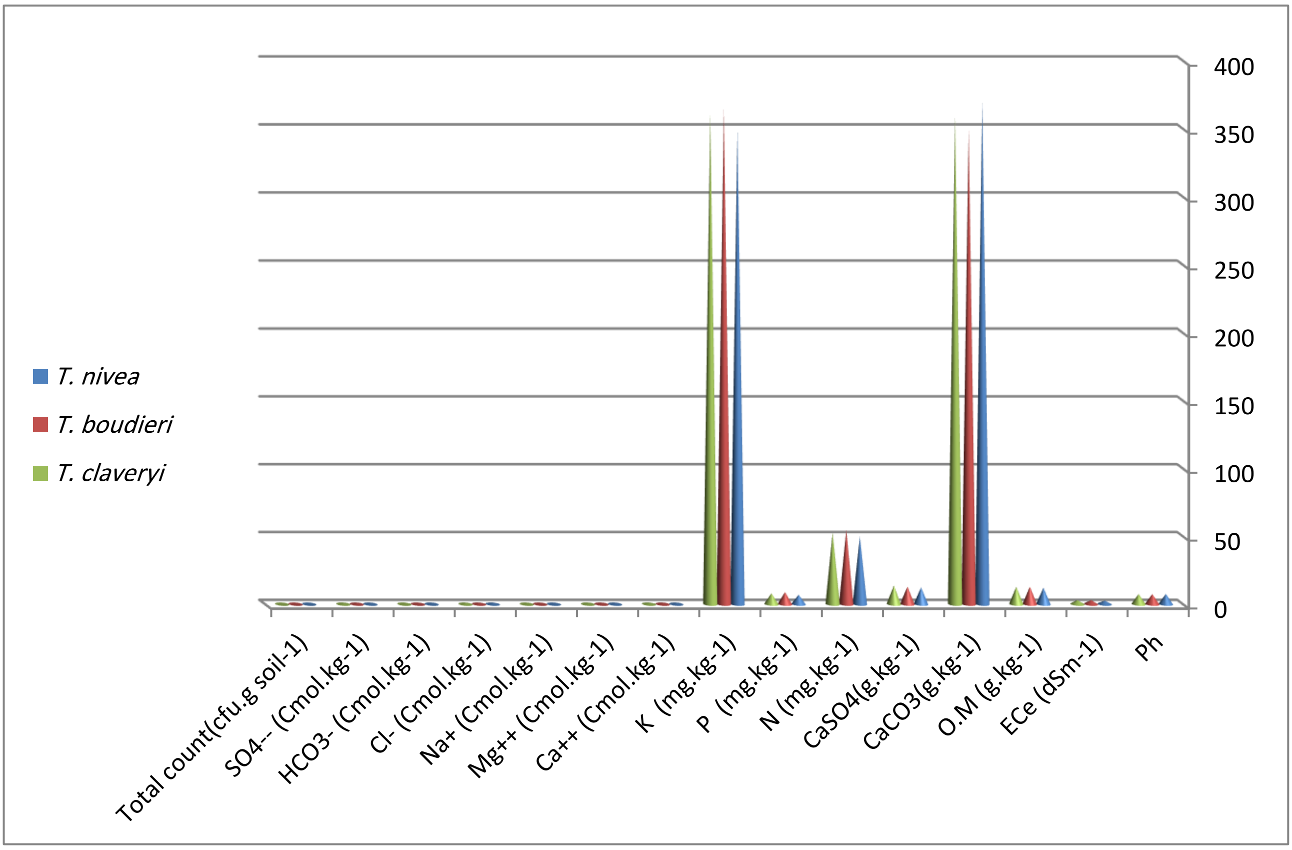
RESULTS AND DISCUSSION:

Truffles are consumed for their special taste and applied a condiment. They are also known for their culinary benefits and distinctive aroma. Some species sell for between $1,000 and $3,000 per pound (Lefevre, 2008). Interest in this type of truffle stems from its high nutritional value, rich protein content, and delicious taste (Murcia et al., 2002). It also boasts unique nutritional value, including unsaturated fatty acids, vitamins, minerals, and protein (Pacioni, et al., 2014).The results were as follows The chemical substances in the soil were calculated and the results were according to Table (No. 1). The results showed that calcium carbonate CaCO3 was the highest in all three species, while the proportions of the remaining elements varied. Sodium ions were the lowest in T. nivea, while carbonyl acid was the lowest in the other two species. This is attributed to the nature of the soil and the nutritional needs of the

Table 1. soil identification.

Soil components	***T. nivea***	***T. boudieri***	***T. claveryi***
Ph	7.61	7.46	7.51
ECe (dSm^{-1})	2.66	3.03	2.97
O.M ($g.kg^{-1}$)	12.23	13.02	12.92
$CaCO_3$ ($g.kg^{-1}$)	370.23	350.04	359.85
$CaSO_4$ ($g.kg^{-1}$)	12.52	13.06	13.82
N ($mg.kg^{-1}$)	52	56	54
P ($mg.kg^{-1}$)	8	8	7
K ($mg.kg^{-1}$)	352	366	363
Ca^{++} ($Cmol.kg^{-1}$)	0.62	0.71	0.67
Mg^{++} ($Cmol.kg^{-1}$)	0.51	0.47	0.41
Na^{+} ($Cmol.kg^{-1}$)	0.25	0.31	0.27
Cl^{-} ($Cmol.kg^{-1}$)	0.72	0.76	0.77
HCO_3^{-} ($Cmol.kg^{-1}$)	0.36	0.24	0.26
SO_4^{--}	0.56	0.58	0.58
Total count(cfu.g $soil^{-1}$)	5.4*1010	2.4*1010	3.5*1010

Figure 1. soil identification.



This affects the chemical composition surrounding the truffles. The bacterial and fungal biomass ranged from 0.106 to 9.080, depending on the methodology used. The recorded proportions of (fungi + bacteria) ranged from 0.39% to 54.78% and 0.25% to 45.05%, respectively. While these proportions varied, this indicates that bacteria contribute more significantly to soil ecosystems. The relative contribution of bacteria and fungi can be better assessed using the fungal uptake:bacteria ratio, rather than the biomass or production ratio. However, variability in estimates of microbial production and uptake persists due to their complex responses to soil biological activities, as well as the number of fungi and bacteria associated with truffle roots. The protein of all types of desert truffles contains amino acids in varying amounts, with the highest value being in the desert truffle T. nivea, at 556.365 parts per million, while it reached 346.633 and 239.609 parts per million in the desert truffle T. claveryi and T. boudieri respectively (Tables 2, 3 and 4). The T. nivea truffle contains the fewest amino acids: aspartic acid, serine, threonine, alanine, cysteine, methionine, isoleucine, and lysine. In contrast, the T. boudieri truffle contains the highest number of amino acids, up to 14: arginine, alanine, cysteine, valine, methionine, phenylalanine, isoleucine, and leucine. This is followed by the T. claveryi truffle, which contains 11 amino acids: aspartic acid, glutamic acid, and serine. Of the three species (aspartic acid, glutamic acid, serine, histidine, glycine, threonine, histidine, glycine, cysteine, valine, phenylalanine, isoleucine, and leucine), the results show that all three desert truffle species contain a significant amount of important amino acids Food (Bedade et al., 2012). It is believed that the variation in the quantity and quality of amino acids among the species is due to differences in the environments associated with truffles, as truffles are influenced by surrounding environmental conditions (Belfiori et al., 2016). Aspartic acid, serine, cysteine, and isoleucine are also present in all three species. This similarity in these three amino acids may be attributed to the genetic similarity of the three truffle species, as recent studies have indicated a high degree of similarity in gene pairs (Benucci et al., 2016). T. boudieri truffles are distinguished from the other species by their arginine content, while T. nivea and T. claveryi contain lysine.

Table (2) The amount of amino acids in *T. nivea*

Reten.Tim(min)	Response	Amount(ppm)	Amount(%)	Amino acid
9.084	982.124	40.525	7.3	Aspartic acid
11.832	3468.308	35.480	6.4	Serine
12.568	1184.302	12.866	2.3	Threonine
12.880	481.861	0.000	0.0	---------
13.016	7022.729	0.000	0.0	---------
13.328	30646.076	233.555	42.0	alanine
15.936	6284.047	104.309	18.7	---------

Reten.Tim(min)	Response	Amount(ppm)	Amount(%)	Amino acid
16.864	767.440	0.000	0.0	methionine
17.604	2231.464	119.008	21.4	----------
17.824	40.709	0.000	0.0	----------
18.336	6.350	0.294	0.1	----------
18.596	884.662	0.000	0.0	Isoleucine
19.108	266.959	10.327	1.9	Leucine
Total		556.365	100.0	

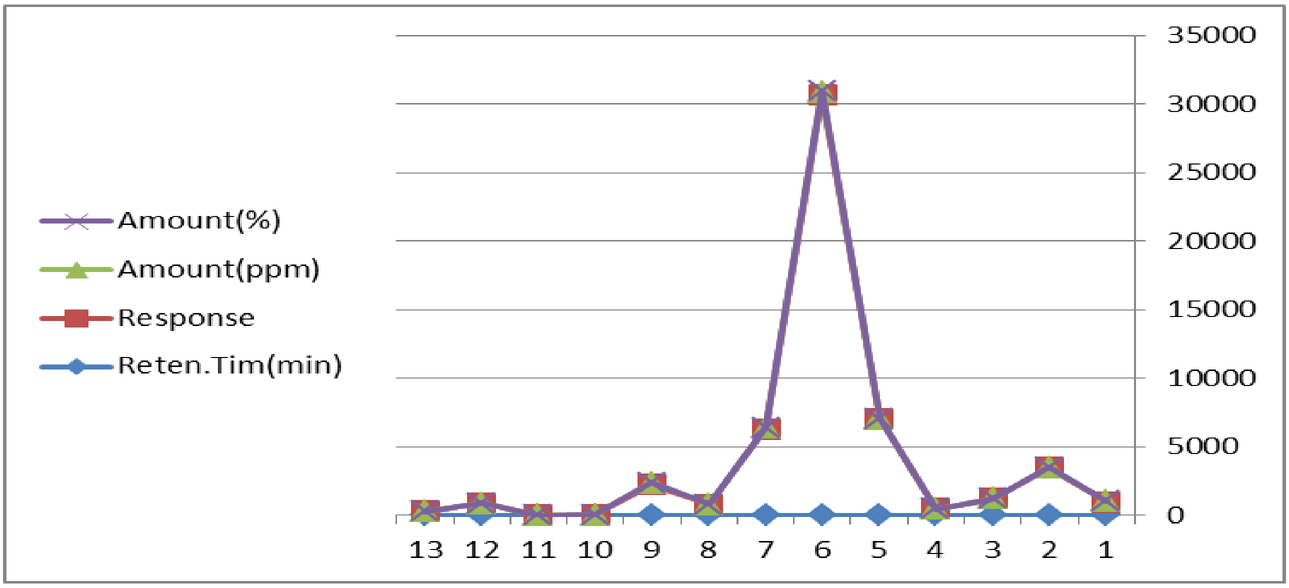


Figure 2. The number of amino acids in *T. nivea*

Table 3. The amount of amino acids in T. claveryi

Reten. Tim (min)	Response	Amount(ppm)	Amount(%)	Amino acid
9.036	4.648	0.192	0.1	Aspartic acid
10.768	6525.148	194.433	56.1	Glutamic acid
11.841	1920.948	19.651	5.7	Serine
12.020	3008.521	13.789	4.0	Histidine
12.425	2393.080	16.023	4.6	Glycine
12.524	433.823	0.000	0.0	---------
16.144	646.865	10.737	3.1	Cystine
16.460	11729.612	0.000	0.0	---------
16.787	1546.907	0.000	0.0	----------
17.212	1030.078	21.878	6.3	Valine

Reten. Tim (min)	Response	Amount(ppm)	Amount(%)	Amino acid
17.332	314.236	0.000	0.0	----------
17.956	375.006	12.180	3.5	Phenylalanine
18.548	1111.660	51.549	14.9	Isoleucine
18.780	139.757	3.098	0.9	Leucine
18.992	80.223	3.103	0.9	Lysine
Total		346.633	100.0	

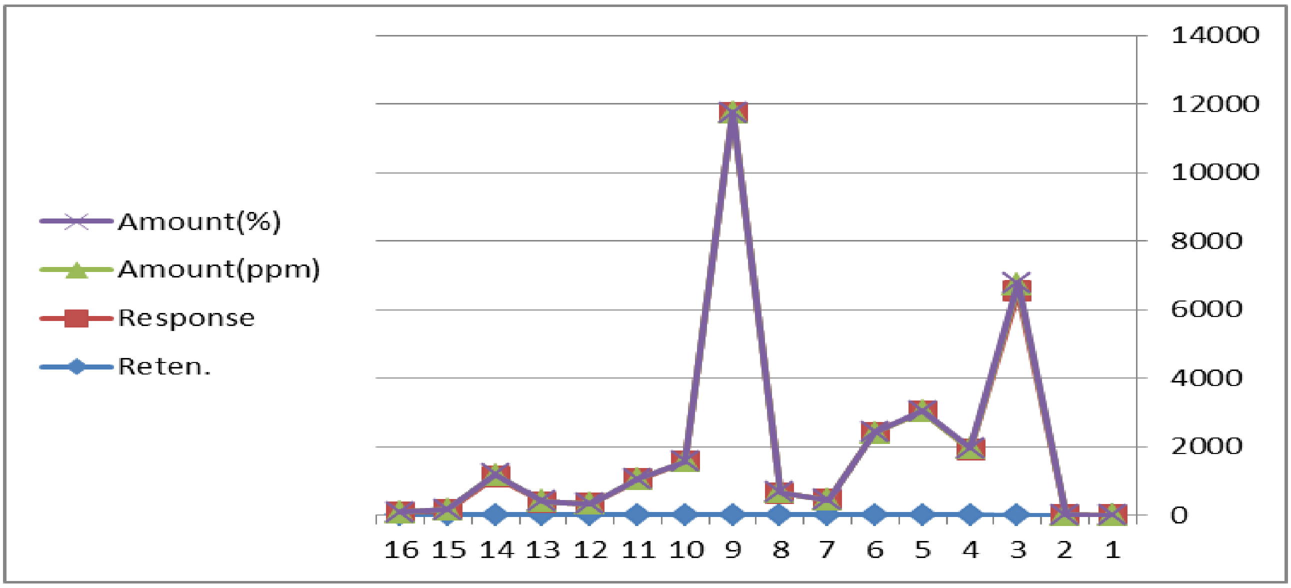


Figure 3. The number of amino acids in *T. claveryi*

Table 4 The number of amino acids in *T. boudieri*

Reten. Tim(min)	Response	Amount(ppm)	Amount(%)	Amino acid
8.952	0.276	0.011	0.0	Aspartic acid
10.820	2663.477	79.365	33.1	Glutamic acid
11.904	1210.757	12.386	5.2	Serine
12.068	1327.437	6.084	2.5	Histidine
12.480	1873.428	12.543	5.2	Glycine
12.724	1704.079	18.513	7.7	Threonine
13.052	5827.920	0.000	0.0	----------
13.084	2240.735	0.000	0.0	----------
13.152	4356.773	75.654	31.6	Arginine
13.412	1283.819	9.784	4.1	Alanine
15.928	24.403	0.405	0.2	Cystine

Reten. Tim(min)	Response	Amount(ppm)	Amount(%)	Amino acid
17.288	581.560	12.352	5.2	Valine
17.496	66.521	3.548	1.5	Methionine
18.084	38.137	1.239	0.5	Phenylalanine
18.600	101.893	4.725	2.0	Isoleucine
18.704	556.584	0.000	0.0	------------
18.872	135.355	3.000	1.3	Leucine
Total		239.609	100.0	-----------

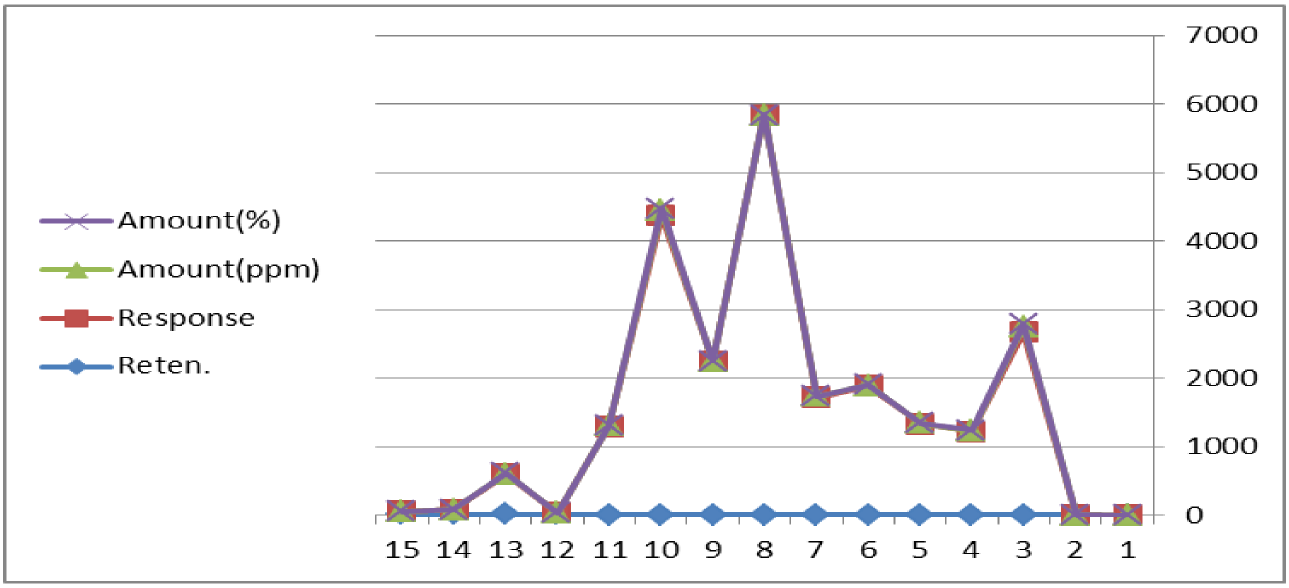


Figure 4. The number of amino acids in *T. boudieri*

The results, as shown in the (table 2,3,4) indicate a difference in the protein composition of the three truffle varieties. This is evident in the type and proportion of amino acids in each protein. This difference is due to the genetic makeup of these varieties. Previous studies have shown that the sequence of nucleic acids varies among the three varieties. This difference is significantly reflected in the external appearance, nutritional value, and resistance of these varieties to storage conditions. These conditions combined determine the price value of each variety.

CONCLUSIONS

These subterranean ascomycetes are in high demand in the culinary and culinary worlds; however, they are enjoyed by only a few due to their limited availability and high cost. Given their rich nutritional content, There are no recorded side effects, which adds value to the diet, efforts should be directed toward exploring methods for culturing them, intensifying research into their genetic characteristics, and developing methods that facilitate their propagation so that they are available to the largest possible population at affordable prices.

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