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Biochemical and Health Properties of Truffles

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ABSTRACT

Truffles are the most expensive edible mushrooms refer to genus *tuber* which grows symbiotically in plant roots such as oaks and hazels. Truffles are underground mushrooms also known for their characteristic earthy flavor which is the major reason for their special place in the culinary. Their characteristic intense aroma helps them in reproduction by attracting small animals. Truffles can survive in a wide range of environments such as deep forests as well as deserts. The most expensive varieties of truffles include *Tuber melanosporum* (Black truffle), *Tuber magnatum* (White truffle), *Tuber aestivum* (Burgundy truffle), *Tirmania nivea*, and *Terfezia chlaveryi* (Dessert truffles). Truffles vary in their composition and flavor profile from species to species. The major volatile components which are responsible for truffle aroma are aldehydes, ketones, sulfur compounds, alcohols, and esters. Truffles are highly nutritious, rich in antioxidants, and have therapeutic properties such as antimicrobial activity, antiviral activity, antimutagenic activity, anti-inflammatory activity, hepatoprotective activity, etc. The major active components present in truffle are tuberoside, phenolics, anandamide, and ergosterol.

Keywords: Truffles; Mushroom; Health; Nutrition; Antioxidant

1. INTRODUCTION

Truffles are edible mushrooms from the genus Tuber. They are hypogeous subterranean ectomycorrhizal ascomycetous fungi that form a symbiotic relationship with plant roots[1]. They are found in a variety of habitats including temperate forests, Mediterranean woodlands, subtropical cloud forests, flood plains, boreal forests, tree nurseries, etc. Truffles are mostly found in temperate zones of the northern hemisphere. Truffles are widely known for their characteristic aroma and taste. Their intense aroma helps them in spreading their spores. Rodents and animals such as dogs, foxes, hogs, etc. find truffles by their attractive aroma and feed truffles then spread their spores. Truffles don't have any specific shape; usually, it looks like a potato. Size varies from 3 cm - 15 cm and weight varies from 5 g - 200 g. Among the different varieties of truffles most precious are the scented ones such as *Tuber melanosporum* Vittad (Perigord black truffle) and *Tuber magnatum* Pico (white truffle). *T. aestivum* also known as burgundy truffle is another species widely known for its delicacy. Tuber species forms a strong association with host plants as well as soil. Plants such as *Fagus sylvatica* (beech), *Betula* (birch), Hazelnut (*Corylus avellana*), and Oak (*Quercus robur*) are usually found in symbiosis (Fig. 1). Truffles grow at various altitudes and mostly in calcareous soils with a sub-alkaline pH (7-8)[2-3]. Along with its unique flavor, it also has therapeutic properties so that it can be used as a functional food ingredient.

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Figure 1. Truffle ground with a host plant[6].

2. VARIETIES /BIODIVERSITY OF TRUFFLES

Thousands of varieties of truffles are grown over the whole world and all of them are not edible. Only those from the genus *Tuber* are used as a food source. Out of these edible truffles, black and white truffles are well known for their unique and delicate flavor (Fig. 2). Edible truffle species in which more research is going on include *T. melanosporum* (black truffle), *T. brumale* (black truffle), *T. aestivum* (black truffle), *T. indicum* (black truffle), *T. magnetum* (white truffle), *T. borchii* (white truffle), *Tirmania nivea* (white truffle), *Terfezia* (black truffle),

Figure 2. A Black truffle from East Africa[34].

which will elicit stress and defense responses in surrounding fungi, bacteria, and especially in herbaceous plants.

Buntgen *et al.* conducted a study to find out the relation between size and maturity of Burgundy truffles *(T. aestivum)* and found that there is no strong relation between fruit body weight and maturity[7]. They reported that the presence of small-ripe and large-unripe fruiting bodies. The study predicted maturity can be depending on the surrounding ecosystem which includes soil, climate environment as well as the microbiome, and mycelial connectivity.

3. NUTRITIVE VALUE OF TRUFFLES

Nutritional profiles of truffles are influenced by several factors such as species, geographical area, type and pH of the soil, etc. Fresh truffles contain a moisture content of 77.1 per cent, protein 7.6 per cent, lipid 0.5 per cent, ash 1.9 per cent, and crude fiber 6.4 per cent along with calcium phosphorus and trace amounts of micro minerals (manganese, aluminum, iodine)[8]. Dessert truffles are rich in potassium, phosphates, and significant amounts of iron. Dry matter consists of 20 per cent - 27 per cent of protein, 3-7.5 per cent of fat, 7 per cent - 13 per cent crude fiber, and also rich in ascorbic acid i.e., around 2-5%[9]. The nutritive value of some edible truffles is given in Table 2.

Table 1. Geographical distribution of truffles

Truffles	Geographical area	Reference
T. melanosporum (black truffle)	Found in Mediterranean regions of France, Italy, parts of Croatia and Serbia, the greater part of northern Spain	10, 12, 18
T. aestivum (black truffle, Summer truffle)	Northern Italy and parts of France	10,12 18, 25
Terfezia claveryi (black truffle)	Middle East countries, the Mediterranean basin, North Africa, and Kalahari desert in Botswana.	12, 19, 20, 23, 35
Tirmania nivea (white truffle)	Middle East countries, the Mediterranean basin, North Africa, and Kalahari desert in Botswana.	12, 19, 22, 26
T.borchii (white truffle)	Found throughout Europe especially Italy	10, 12
T.magnetum (white truffle)	Found in Italian region such as piedmont, Tuscany, Emiglia Romagna, and in a small region of southern Switzerland	10, 12

Table 2. Nutritional value of truffles

Truffles	Nutritive values	Reference
T. melanosporum	8.7g soluble protein and total carbohydrate content of 1.77 g.	
T. magnetum	11.0g soluble protein and total carbohydrate content of 5.65 g.	10
T. aestivum	The total carbohydrate of 5.65g and soluble protein 11.0 g	
Tirmania nivea	75.27 % moisture, 27.18 % protein, 7.42 % fat, 13.02 % crude fibre and 5.4% ash.	
Terfezia claveryi (black truffle)	75.44 % moisture, 24.96 % protein , 4.2 % fat, 7.02 % crude fibre and 6.39 % ash (on dry weight basis)	30

etc (Table 1). Truffles that grow in arid and semi-arid areas are known as desert truffles especially found in Mediterranean areas such as *Terfezia* and *Tirmania* species[4-5].

Zampieri *et al.* conducted a metaproteomics study in the soil where black truffles are grown around a host plant[6]. He found that the environment in the truffle ground is very active as compared to the outside and the area was dominated by broad stress responses from its components mainly from host plants. Truffles produce a wide range of volatile metabolites

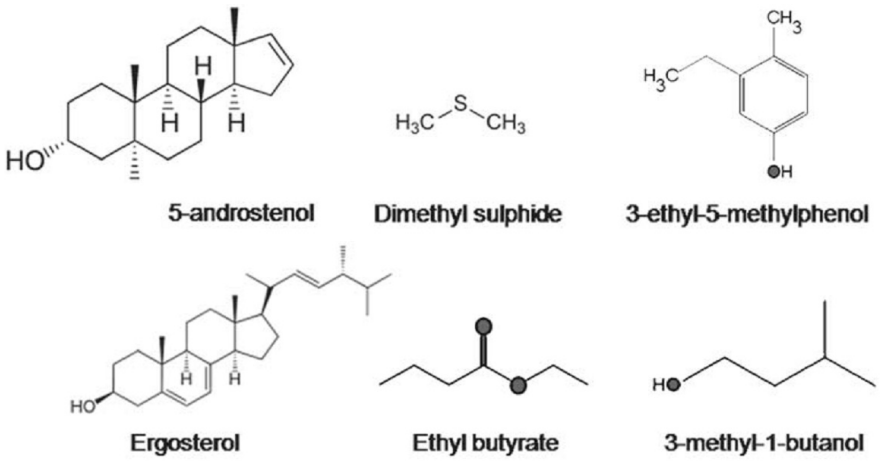

Figure 3. Some aromatic compounds of truffles[35].

Table 3. The flavor profile of truffles

Truffles	Flavor components	Reference
T. melanosporum (black truffle)	3-Ethyl-5-methyl phenol, 5-methyl-2-propyl phenol, B-Phenylethanol, 3-Ethylphenol	10, 12, 18
T. aestivum (black truffle, Summer truffle)	Dimethyl sulfide, B-Phenylethanol, 3-Ethylphenol	10, 12, 18, 25
Tirmania nivea (white truffle)	Hexadecanoic acid,11, 14-eicosadienoic acid methyl ester, octadecanoic acid	12, 22, 25
T. magnetum (white truffle)	Bis (methylthio) methane, Dimethyl sulfide, Dimethyl disulfide	10, 12

Compared to other types of edible mushrooms truffles have higher protein content and sulfur-containing amino acids such as cysteine, methionine, and lysine. According to Saltarelli *et al.*, when comparing protein content of four species *T. magnatum, T. melanosporum, T. aestivum,* and *T. borchii*, a higher amount was found in *T. magnatum* and *T. melanosporum* ranging from 8.7 per cent to 24 per cent on a dry weight basis[10]. Truffles are a good source of minerals such as potassium, phosphorous, iron, sodium, magnesium, manganese, zinc, and calcium. Because of its costly nature even if it is highly nutritious it's not suitable as a part of human's daily diet[11].

4. FLAVOUR PROFILE OF TRUFFLES

Truffles are well known for their unique and delightful aroma and delicate taste that is responsible for their wide popularity. This odorant helps them to attract rodents and mammals which feed the mycelia and help them to spread their spores. Truffles vary in their characteristic aroma from species to species it can be expressed as earthy, pungent, musky, nutty etc[12]. Hundreds of aromatic compounds are identified in truffles and studies are going on to reveal the contribution of each of these compounds to the aroma of truffles (Fig. 3). To find aromatic compounds several techniques are used such as Head-space solid-phase micro-extraction, GC-MS analysis, Proton transfer reaction mass spectrometry, and gas chromatography-olfactometry. Major aromatic compounds in truffles are dimethyl sulphide, Dimethyl disulphide, 2, 3-butanedione, Ethyl butyrate, 1-hexen-3-one, 3-methyl-1-butanol, methional, 3-ethyl-5-methyl phenol (Table 3) .

Vahdatzadeh *et al.* studied the role of the microbiome in the generation of aromatic components. Microbes are present in truffles at all stages of their life cycle from fruiting bodies to the free-living mycelial stage, which includes bacteria, yeast, and viruses[13]. Aromatic compounds include sulfur compounds, alcohols, esters, ketones, and aldehydes. Dimethyl sulfide is a major odorant present in fungal mycelia and *Alpha-proteobacteria* which is the dominant microbe in the fruiting body is also capable of producing dimethyl sulfide. Microbiome which colonised fungal mycelia also possess the capacity to produce aromatic components but their role is not yet clearly confirmed.

Kamle *et al.* conducted a study on dessert truffles *Tirmania nivea, Picoa lefebveri*, and *Terfezia boudieri* and identified volatile components responsible for their aroma[14]. Major volatile components in *T. boudieri* and *T. nivea* were found that 1-octen-3-ol and hexanal. *Picoa lefebvrei* was found to contain components with less volatility.

5. PRESERVATION OF TRUFFLES

Preservation methods for truffles are selected based on their flavor profile. The selection of the preserving method is very important in the case of truffles to preserve flavor components without any loss during storage time[15]. Low-temperature preservation methods are widely accepted for preserving truffles without much biochemical spoilage (Table 4). According to Saltarelli *et al.*, during refrigeration white truffles (*T. magnatum* and *T. borchii*) are more sensitive to spoilage than black truffles (*T. melanosporum* and *T. aestivum*)[10]. Nazzaro *et al.*, suggested that irradiation is an effective method to preserve fresh black truffles with lesser biochemical alterations especially suitable for decontamination of truffles[15]. Low dose irradiation in the range of 1.5 KGy was found to be best as compared to other doses to decontaminate truffles[16]. According to studies conducted by Rivera *et al.*, fresh black truffles (*T. melanosporum and T. aestivum*) can be stored without losing their quality with the help of modified atmosphere packaging (MAP)[17]. MAP with microperforated films can extend the shelf life of truffles by storing them in a mixture of 15 per cent CO2 and 7 per cent O2. Combined treatments can also provide some synergistic effects such as a combination of gamma irradiation, acid and alkali treatment, and refrigeration. A study on *T. aestivum* shows that a combination of refrigeration, microperforated plastic films, and electron beam irradiation can improve the shelf life by two times without the development of mycelium and yeast growth[18-19].

Table 4. Methods for the preservation of truffles

Truffles	Type of preservation	Reference
T. melanosporum (black truffle)	Refrigeration, MAP with micro-perforated films	10, 12, 18,
T. aestivum (black truffle, Summer truffle)	Refrigeration, MAP with micro-perforated films, Combination of Micro perforated films with electron beam irradiation, and refrigeration	10, 12, 18, 25
Terfezia claveryi (black truffle)	Combination of blanching in 4 % boiling Nacl, spraying with 5 % vinegar, freezing at -18 °C, dry in an oven at 110 °C. Combination of gamma radiation, acid, and alkali treatment, and refrigeration	10, 12 19, 23, 35
Tirmania nivea (white truffle)	Combination of blanching in 4 % boiling Nacl, spraying with 5 % vinegar, freezing at -18 °C, dry in an oven at 110 °C. Combination of gamma radiation, acid, and alkali treatment, and refrigeration	12, 19, 22, 26
T. borchii (white truffle)	Refrigeration	10, 12
T. magnetum (white truffle)	Refrigeration	10, 12

MAP = Modified atmosphere packaging

Table 5. Biological functions of truffles

Truffles	Biological functions	Reference
T. aestivum (black truffle, Summer truffle)	Antimutagenic properties	10, 12, 18, 25
Terfezia claveryi (black truffle)	Hepatotoxicity, Antioxidant activity, Antiviral, and Antimicrobial activity	12, 19, 20, 23, 35
Tirmania nivea (white truffle)	Antioxidant activity, Antiviral and Antimicrobial activity	12, 19, 22, 26
Elaphomyces granulatus	Anti-inflammatory activity, Antioxidant activity	12, 27

6. BIOCHEMICAL PROPERTIES OF TRUFFLES

Truffles are well known for their unique flavor and nutritional value in addition to that few bio-activity parameters are also reported in truffles such as antioxidant activity, antimicrobial activity, antiviral activity, antimutagenic activity, anti-inflammatory activity, and hepatoprotective activity etc.[20-26]. Stanikunaite *et al.*, carried out a study in which twenty-two species of hypogeous truffles and other fungi similar to truffles are screened for bioactivities[24]. The study revealed the therapeutic potential of truffles in terms of six biological activities antimicrobial, antimalarial, anti-inflammatory, antioxidant, anti-tuberculoid, and anticarcinogenic activity (Table 5). Stanikunaite *et al.*, conducted a study on *Elaphomyces granulates* to reveal bioactive components which are responsible for anti-inflammatory activity[27]. They found that syringaldehyde and syringic acid isolated from *E. granulates* exhibited cox 2 inhibitions which have an important role in inflammation.

Antimutagenic properties are reported in fresh and irradiated black truffles (*T. aestivum*). A study was conducted in *Salmonella typhimurium* against 2-aminothracine and sodium azide. Fresh truffle exhibited strong antimutagenic properties than irradiated one[25]. Hepatoprotective activity was observed in *T. claveryi* as a result of a study conducted against CCl_4 [28].

Antioxidant activity was reported in many truffle species such as *Terfezia claveyichatin, Picoa juniper vittadin, Tirmania nivea, Elaphomyces granulatus*, etc. According to a study conducted by Stanikunaite *et al.*, syringic acid is responsible for the strong antioxidant activity of *E. granulatus*[27]. *T. nivea*, dessert truffle from different places were analysed for antioxidant capacity in terms of DPPH, FRAP, Nitric oxide scavenging, and inhibition of deoxyribose breakage. The study, also found that *T. nivea* species contains a significant amount of ascorbic acid, phenolic content, flavonoids, and total carotenoid content[26].

Murcia *et al.*, state that when comparing *Terfezia claveryichatin and Picoa juniper vittadin* with common antioxidants such as BHA, BHT, and α-tocopherol, it showed higher antioxidant potential[23]. They conducted assays such as lipid peroxidation, deoxyribose, and peroxidase, and canned and frozen samples which showed a reduction in radical scavenging potential as compared to a fresh one.

Janakat *et al.*, and Hussan and Al-Ruqaie studied antiviral and antimicrobial activities in *T. nivea* and *T. claveryi* and found that *Tirmania* is less effective when compared to *Terfezia* in antibacterial activity[20,22]. Extracts of these species are effective against a wide range of gram-positive and gram-negative bacteria including *Bacilussubtilis, Staphylococcus aureus,* and *Chlamydia trachomatis.*

7. SOME COMMON VARIETIES OF TRUFFLES

7.1 *T. Melanosporum* (Black Truffle)

Tuber melanosporum Vittad ascocarps of genus *Tuber* also known as black truffle, Perigord truffle, and French truffle etc[5,29]. *T. melanosporum* truffles weigh up to 100 g when fully matured and with an average diameter of 7 cm. Perigord truffles are native to the Mediterranean regions of France, Italy, and the larger part of northern Spain. It's harvested from the end of November to starting of March. Black truffles grow well in calcareous soil with a pH above 7.5. Oak and lime are the two mostly hosted plants for their growth[4-5]. Saltarelli *et al.*, analysed *T. melanosporum* truffles and found that it contains 8.7 g ± 0.83 g soluble protein and total carbohydrate content of 1.77 g[10]. The aroma profile of *T. melanosporum* truffles shows that 3-ethyl-5-methyl phenol, 5-methyl-2-propyl phenol, B-Phenyl ethanol, and 3-Ethylphenol are the dominant compounds that are responsible for the unique flavor of truffles.

7.2 *T. Magnetum* (White Truffle)

T. magnatum truffles are also known as Piedmont white truffles, native to the Italian region especially Piedmont, Romagna, Emiglia, and some regions of southern Switzerland. It grows to a diameter of 12 cm with a weight of 500 g. It is harvested during the winter season from mid-October to the end of January. Piedmont truffles grow in soft, moist soil rich in calcium carbonate. Alder, Poplar, Oak, and hazel are the common host plants for piedmont white truffles[4-5]. Saltarelli *et al.*, analyzed the nutritional properties of *T. aestivum* truffles and found that it contains 11.0g±0.93g soluble protein and total carbohydrate content of 5.65 g[10]. Bis (methylthio) methane and Tris (methylthio) methane are the two dominant flavor compounds available in *T. magnatum* truffles.

7.3 *T. Aestivum* (Black Truffle, Summer Truffle)

T. aestivum truffles are brown to black they grow to a maximum diameter of 10 cm. They are found in northern Italy and parts of France and they are harvested in September or late December or the end of January. Summer truffles grow in soil that is very stony and rich in organic matter. Common host plants are beech, birch, hazelnut, and English oak[4-5]. Saltarelli *et al.*, conducted a study in *T. aestivum* truffles and found that it contains a total carbohydrate of 5.65 g and soluble protein 11.0 g± 0.93 g[10]. Dimethyl sulfide, B-phenyl ethanol, and 3-ethylphenol are the dominant flavor components present in *T. aestivum* truffles.

7.4 *Termania Nivea* (White Truffle)

Termania nivea is a type of desert truffle which grows in dry areas with sandy soil. Desert truffles are native to the Middle East also found in the Mediterranean basin, North Africa, and the Kalahari Desert in Botswana. They will grow up to a diameter of 10 cm and more and weigh up to 1 kg. It is whitish-yellow with a smooth structure and warts. It is harvested from February to April. Common host plants are rock rose trees, camel thorn, and helianthemum[5,30]. Swaya *et al.*, analysed nutritional properties of unpeeled *Tirmania nivea* truffles and found that on a dry weight basis it contains 75.27 per cent, protein 27.18 ± 0.04 %, fat 7.42 ± 0.03 %, crude fiber 13.02 ± 0.02%, and ash 5.4 ± 0.08 %[30]. Hexadecanoic acid, 11,14-eicosadienoic acid methyl ester and octadecanoic acid are the dominant flavour components present.

7.5 *Terfezia Claveryi* (Black Truffle)

Terfezia truffles are black truffles that grow in desserts. They grow to a maximum diameter of 10 cm and weigh up to 1 kg. They are found in the Middle East, the Mediterranean basin, North Africa, and the Kalahari Desert in Botswana. They are harvested from February to April. *Terfezia* truffles grow in mildly acidic dry sandy soil with a rainfall of 200 mm - 250 mm[5,30]. According to Sawaya *et al.* unpeeled *Terfezia claveryi* truffles contains 75.44 per cent of moisture, 24.96 ± 0.04 % protein, 4.2 ± 0.31 % fat, 7.02 ± 0.22 % crude fiber, and 6.39 ± 0.36 % ash on a dry weight basis[30].

8. TRUFFLE PRODUCTS

There are several types of products are available in the market made from truffles such as truffle spread, truffle honey, truffle pesto, truffle nut mix, truffle sea salt, white truffle mayonnaise, black truffle rub, truffle butter, porcini and white truffle sauce, black truffle popcorn, etc (Fig. 4).

Figure 4. Truffle products available in the market[36].

Black truffle popcorn is nothing but popcorn coated with virgin olive oil, truffle, and sea salt and sold under the brand name Joe and sephsin England. White truffle sea salt is made by mixing white truffle shavings and sea salt. Which can be used in sauces and meat marinades to impart a characteristic truffle flavor. White truffle sea salt is famous for the Daylesford brand.

Marks and Spencer brand brought a new truffle product, truffle pesto to the market. Truffle pesto is a combination of olive oil, cashew nuts, cheese, and black truffle. Harvey Nichols brand has developed truffle honey which contains delicate acacia honey with a floating small piece of dried *bianchetto* truffle. This honey is full of truffle flavor and a swirl of this can be used in food products to impart truffle flavor e.g. glazing of meat. Harvey Nichols truffle spread is another unique product that contains cheese and *bianchetto* truffle from Italy. Truffles are also available in the form of slices in the market, which are packed in olive oil to preserve their freshness and flavor e.g. black truffle slices in olive oil from the brand of selezione in Italy.

9. STANDARDS FOR REGULATING TRUFFLES TRADE

Regulations related to truffle usage are very limited. Truffles outer skin is reported to contain chitin and melanin. Melanin is responsible for the dark color of the ascocarp of black truffles. The after-effects of its long-time usage are still not yet studied[31]. Balestrini and bonfante reported that Chitin is a major component of truffle cell wall[32]. Chitinolytic bacteria such as *Pseudomonas fluores*cence and *Bacillus sp.* are also found in cell walls as sporocarps[33]. Codex Alimentarius commission made regulations for standardizing the quality of truffles trade. They have revised their standards related to truffles recently in April 2016 (CAC/39 INF/8).

United Nations Economic Commission for Europe (UNECE) developed standards concerning marketing and commercial quality control of truffles. These standards are only applied to truffles that are supplied to the consumer as fresh and industrially processed are excluded. According to standard fresh truffles supplied to the consumer should be intact, firm, sound, clean, and free from pest damage and abnormal external moisture. According to UNECE standards, truffles are classified into three Classes i.e., Extra class, Class I, and Class II. Truffles from the extra class must be of superior quality, round in shape, and with exception of slight superficial defects. Class, I truffles are of good quality with a slight defect in appearance and slight superficial bruising. In the case of class II truffles defects are allowed provided that they should retain their essential characteristics regards keeping quality and presentation. Defects can be in shape, development, and coloring. Extra class truffles will be weighing around 20 g and class I will be 10 g and class II is 5 g (UNECE standard FFV-53, 2017).

10. CONCLUSION

Truffles are underground treasures that have many advantages over other edible mushrooms. They have proved their value in all aspects ranging from the culinary field to its therapeutic usages. Even though they are rich in the nutritive value we are unable to make them a part of the daily human diet because of rare availability, costly nature, etc. The major problem faced during truffle processing is the preservation of its flavor and aroma. Still, there is a gap in the preservation technologies that can maintain flavor and aroma throughout its shelf life. Truffles vary widely from species to species in their composition, aroma components, and biological activities. Still, more research to be conducted to develop value-added products, to identify active components and preservation technologies, etc.

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