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REVIEW ARTICLE

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Tour of Truffles: Aromas, Aphrodisiacs, Adaptogens, and More

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ABSTRACT

Truffles are the fruiting bodies of ascomycete fungi that form underground. Truffles are globally valued, culturally celebrated as aphrodisiacs, and highly sought-after delicacies in the culinary world. For centuries, naturalists have speculated about their mode of formation, and in cultures surrounding the Mediterranean Sea, many species have been prized as a delectable food source. Truffle fruiting bodies form underground and emit a variety of volatile organic compounds (VOCs). Truffle volatiles are believed to have evolved to attract animals that disperse their spores. The main VOCs identified from truffles include sulfur compounds, such as dimethyl sulfide (DMS) and dimethyl disulfide (DMDS); in addition, 1-octen-3-ol and 2-methyl-1-propanol have been found in most truffle species. Humans use pigs and dogs trained to detect truffle VOCs in order to find these prized subterranean macrofungi. Truffles have pharmacological potential, but until more reliable cultivation methods become available their high price means they are unlikely to see widespread use as medicinals.

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Introduction

Truffles are the subterranean reproductive structures of hundreds of species of fungi belonging to the class Ascomycetes. Gourmets value a few species of truffles for their exquisite flavor and aroma. They are also purported to be aphrodisiacs. In particular, two species, *Tuber magnatum*, the "white truffle," and *Tuber melanosporum*, the "black truffle," are considered among the most delectable of all edible fungi. In recent years, the yield of truffles has dropped while the demand has grown, leading to extremely high prices [1]. Depending on the species, 1 kg of truffles can range from €600 to €6000 [2,3]. In 2010, a single, large (900 g) white truffle was sold to Jeannie Cho Lee, a South Korean wine critic living in Hong Kong, for $144,000 [4]. Gourmet truffles cost so much that they have gained monikers like "underground gold" and "diamonds of the kitchen" [5,6]. One online gourmet site pointed out that truffles now cost more than cocaine [7].

In this brief "tour of truffles," we review the history, taxonomy, and collection of these interesting hypogenous fungi; describe the chemistry, flavor, and purported aphrodisiacal properties of their aroma compounds; summarize contemporary research on the physiological activity of truffle metabolites; and conclude with some material about the promised medical potential of their natural products.

History

As early as 1600 B.C. an anonymous source described truffles as "mysterious products of the earth." The Greek philosopher Theophrastus (c. 370 – 286 BCE) wrote descriptions of truffles in *Historia plantarum* regarding their reproduction and physiology, and the Roman orator Cicero (106 – 43 BCE) called truffles the "children of the earth." The Greek physician Dioscorides (CE 40 – 90) classified truffles as "tuberous roots" [8]. Pliny the Elder (also called Gaius Plinius Secundus, CE 24 – 79) and other Greco-Roman writers associated the proliferation of truffles with thunderstorms and attributed their appearance to the action of lightening [9–11]. It is believed that many of the truffles described in Greco-Roman times were what we now call "desert truffles" in the genus *Terfezia*. They differ from truffles in the genus *Tuber* in that their odor is not as strong, their flesh is not marbled, and they grow in drier climates. They are called *terfez* by the Arabian people of North Africa and referred to as *kame* in Eastern Asia [11]. It has been hypothesized that the *manna* described in the Hebrew Bible, the food which fed hungry Israelites wandering in the desert,

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was a variety of desert truffle. In the Judeo-Cristian Bible [12], manna is described as follows: "It was white like coriander seed and tasted like wafers made with honey." A contemporary expert on the desert truffles of the Middle East, Elionar Shavit, describes them as having "the texture of a crisp potato, the smell of a bee's wax candle just snuffed out, and the flavor of macadamia nuts" [13,14].

In Western culture, after the fall of the Roman Empire, between the 5th and 12th centuries, little new knowledge of truffles was recorded in written literature. However, in the Middle East, truffles continued to be valued for their medicinal worth. For example, the Prophet Muhammad (CE 570–632) recommended truffles to cure eye disorders. In the tenth century, Islamic philosopher and physician Avicenna (also known as Ibn Sina, CE 980–1037) described truffles as a treatment for various disorders including vomiting, wounds, and weakness, and particularly recommended the juice of desert truffles for common eye inflammations [14,15].

In Europe, truffles reemerged as a valued foodstuff toward the fourteenth century amongst the wealthy in Italy and France. The French mycologist M. G. Malençon claimed that truffles transitioned from being a food source for peasants during the Dark Ages to being a premier food for French high society during the Renaissance [8]. Catherine de Medici of Florence (1519–1589) is often acknowledged as introducing sophisticated culinary techniques to France after she married the Duke of Orleans, the future French King Henry II (1519–1559) [8]. Though contemporary scholars of food history credit her personal influence as part of a wider truffle mythology, all historians can agree that French appreciation for truffles grew largely from Italian influences [16]. The "golden age of truffles" began during the 1800s under the influence of French gastronomy.

Early botanists were confused about the origin and reproduction of the truffle fruiting body. The first record of truffles in England dates back to 1693 from Tancred Robinson (1658 – 1748) in a report to the Royal Society [17]. He wrote, "What these Trubs are, neither the Ancients nor Moderns have clearly informed us; some will have them *Callositi*es, or *Warts* bred in the Earth; Others call them subterraneous *Mushrooms*" [17]. Robinson said that this "delicious and luxurious piece of Dainty" was well known in France and Italy and went on to speculate that these English truffles were associated with a thunderstorm: "The Wet swells them, and Lightning may dispose them to send forth their particular Scent so alluring to the Swine" [17].

During the first decade of the eighteenth century, the apothecary and botanist Claude-Joseph Geoffroy (1685–1752) recognized that truffles were fungi, not plants [8]. In 1729, Pier Antonio Micheli (1679–1737), the famous Italian botanist and mycologist, analyzed and defined the description of "seeds" (spores) from truffles and noted their development inside membranous sacs (asci). His observations were debated for over a century and often contrasted with the more whimsical and imaginative theories that supported the fairy tales and stories of the day [8]. One of the first major scientific treatments of truffles, *Monographia tuberacearum*, was published in 1831 by Italian physician and mycologist Carlo Vittadini (1800–1865). The book described 65 truffle species, of which 51 were new. Vittadini was the first to recognize that the fungal-plant root association that we now call ectomycorrhizae was important not only to the truffle but also to the trees. He wrote, "It is our decided opinion, that beyond all doubt the higher plants absorb nutrients from the fungus by their feeder rootlets" [18]. In England, *Tuber aestivum*, the so-called "deer truffle," was collected for many years as a food source, and until World War I, a small number of truffle hunters made their living by collecting these fungi with the aid of trained dogs [11].

Although the United States was not part of the classical tradition of truffle consumption, during the late 19th and then throughout the twentieth century, the country produced a number of outstanding truffle specialists, especially in the Pacific Northwest. The first was Harvey W. Harkness (1821–1901), a physician who became wealthy during the California gold rush and became a good friend of Leland Stanford. Harkness had an earnest interest in mycology and published the first extensive study of North American truffles, *California Hypogeous Fungi* [19], which established the American Pacific coast as a center for truffle research. The tradition continued with the first woman to receive a Ph.D. in Botany from the University of California in Berkeley, Helen M. Gilkey (1886–1972), who over the decades described or revised nearly 70 species and made essential contributions to understanding truffle ecology [20]. Gilkey passed on her valuable collection of fungal specimens to James Martin Trappe (1931–), who arguably became the world's leading expert in truffle biology with over 500 scientific papers and three books on the subject [21]. Throughout his career, he reshaped truffle taxonomy and trained many other mycologists in the field of hypogenous fungi [21].

North America produces many indigenous species with excellent flavors, including the white winter (*Tuber oregonense*) and spring truffles (*Tuber gibbosum*) of the West coast. They grow in mycorrhizal association with Douglas firs. Many American truffle aficionados believe that their taste and aroma equal or surpass that of the Alba truffle. Currently, the truffles of the Pacific Northwest have been studied and documented in more detail than in any other forested region of the world. Information gleaned from North American forest management that encourages truffle formation may help parts of the world like New Zealand and Australia, which are attempting to develop truffle cultivation practices to meet the increasing demand for these delectable macrofungi [18].

Truffle morphology and taxonomy

Unlike commonly consumed mushrooms, truffles do not have stalks or gills, and their mycelium grows underground. The shapes of truffles are often potato-like, hence the genus name *Tuber*. Similarly, the etymological origin of "truffle" comes from the Latin word "tuber" for "lump" derived from *tumere*, meaning to swell as a reference to its globoid shape [6]. They often look like small, dirty, misshapen potatoes and have been termed "the world's homeliest vegetable" [22].

Morphologically, truffle colors range through shades of white, brown, and black, depending on the species, while their surface textures can vary widely from wrinkled, warty, and bruised to smooth or reticulate [6]. The rind is a compact layer composed of thick-walled tissue. Inside, there are elaborate folds or chambers lined with a palisade of asci [23]. The pigments of the black truffles are allomelanins of polyketide origin [24,25]. Mature truffles feel firm, dense, and woody compared to the softer and more fragile textures of common mushrooms [3,8]. The most famous and most valuable truffles are *T. magnatum* Pico, the Piedmont white truffle, and *T. melanosporum* Vittad., the Périgord black truffle. Other well-known species of *Tuber* include the black winter truffle (*Tuber brumale*) that is harvested in Europe from November to March and the *bianchetto* or smaller white truffle (*Tuber borchii*) that resembles the Alba truffle and has a distinctive garlicky aroma. Summer truffles (*T. aestivum*) fruit from May to September across Europe and after harvesting their odor is often enhanced with artificial flavors prior to use in cooking. The Burgundy truffle (*Tuber uncinatum*) is another truffle found throughout Europe. It matures late into the season, earning it the nickname "autumn truffle." The Chinese truffle (*Tuber indicum*) closely resembles the Black Périgord but lacks the flavor and aroma. It is sometimes involved in fraud, so scientists have developed methods for species authentication to distinguish the species [26].

What are "true truffles?" Sometimes culinary purists limit the term "true truffles" to *T. magnatum* Pico, the Piedmont white truffle, *T. melanosporum* Vittad., the Périgord black truffle, and a few other delectable species. More commonly, all members of the genus *Tube*r are described as "true truffles" [27]. However, most contemporary mycologists accept an even broader, more inclusive terminology and embrace all related hypogeous ascomycetes, including the desert truffles. The major taxa of desert truffles are *Terfezia* spp., *Tirmania* spp., and *Mattirolomyces* spp. They are endemic to the semi-arid and arid Mediterranean regions, the Arabian Peninsula, and parts of Africa [28].

Truffles were once classified in the order Tuberales; today, they are placed in the order Pezizales [29]. Laessoe and Hansen [30] present an excellent summary of the history and changing classification schemes surrounding truffle taxonomy and conclude that different types of hypogeous ascomycetes have evolved multiple times.

All truffle species form obligate symbioses as ectomycorrhizae with the roots of both angiosperms and gymnosperms, including a myriad of host plants such as oak, elm, poplar, chestnut, willow, hazel, beech, birch, hemlock, fir, and pine trees, as well as certain shrubs [31]. Ectomycorrhizal symbioses are mutualistic interactions between filamentous fungi and plant roots. In temperate climates, truffles form ectomycorrhizae predominantly with trees, notably with hazels (*Corylus avellana*), oaks (*Quercus* spp.), and pines (*Pinus* spp.). Desert truffles form associations with shrubs, especially rockroses (*Cistus* spp.) [32]. Generally, truffle species prefer warm, fairly dry climates and thrive in calcareous soils, depending on the species [6,33].

During mycorrhizal formation, the fungus coats the host's roots with a mantle of fungal tissue. The fungal hyphae envelop the outer root cells while colonizing the surrounding soil. The hyphae absorb and deliver nutrients to their host plants while the host plant provides the fungus with a residence and carbon source [1]. The fungi also influence the host plants by releasing hormones such as indole-3-acetic acid (IAA) and ethylene [6,34,35].

Truffle hunting and cultivation

Truffle formation is governed by a multitude of dynamic environmental and nutritional factors that are only partly understood. Certain ecological conditions aid in mycelial growth, formation of mycorrhizal associations, and ultimately the formation of the macroscopic truffle. Squirrels, wallabies, mice, and voles, among other animals, play an important role in spore dispersal. Upon consumption of the truffles, the spores are defecated on the ground [36]. Feces concentrate the spores and are more likely to be deposited in a similar area of origin which increases the likelihood that the spores will germinate in a location suitable for establishing a mycorrhiza [36].

Because truffles grow underground and cannot be easily seen, people have traditionally used animals to track and locate truffles by their distinctive aroma. The French often used female pigs because they have a natural instinct to search them out, often attributed to the fact that truffles release a compound similar to the sex pheromone of boars [37]. Nowadays, trained dogs are more commonly used because they are easier to handle than pigs and less likely to consume them [1]. In eighteenth century Germany, the possession of a "Truffelhund" was regarded almost as a badge of nobility; most of these German trufflehunds were poodle species [11]. Today in Italy, a special kind of dog called the Lagotto Romagnolo is used, but almost any breed can be trained to find truffles [38].

It is also possible to find truffles without a pig or dog. For example, truffle hunters sometimes look for a specific species of yellow fly, the *Suillia*, which hover over the ground of overripe truffles [1]. The flies lay eggs on the ground near truffles, thereby providing a food source for their larvae [25]. In some cases, when truffles are ripening, the ones that develop near the surface can be found because they upraise the soil and cause cracks that form short furrows radiating from the center [11].

The Périgord black truffle naturally grows in open woodlands distinguished by the absence of plant life under well-colonized trees. Truffle metabolites eradicate any herbaceous vegetation that may compete with the fungus for surface moisture. The herbicide-like effect of the truffle compounds results in an area of barren soil. In France, these bare areas are referred to as *terre brûlée* (burned ground). In Italy, the spaces are called *bruciata* and signify the presence of the fungal truffle [8]. The *brûlée* tends to appear one to two years prior to the start of truffle production and is a favorable indicator of a tree's ability to produce truffles [1].

The two most sought-after truffles are the Périgord black truffle of France and the Italian white truffle. This white truffle is often considered the most prized of all [8]. They are harvested at different times of the year so they do not usually compete in the market [8]. The Italian white truffle is harvested in various provinces throughout Italy, but the ancient town of Alba located in the Piedmont region is considered one of Europe's most esteemed truffle territories and has been called "the white truffle capital of the world" [8]. The chalk, marble, limestone, and dolomite-rich soil of this region might be the reason why it produces such high-quality truffles [6].

Likewise, the black truffle of Périgord is held with high regard and produced in various regions from Bordeaux in France to Umbria in Italy and parts of Spain; however, the mecca for black truffle production is in Périgueux, France. Stretches of oak and hazelnut orchards implemented for truffle cultivation are known as *truffières* in France and *tartifaie* in Italy [8].

Early in the nineteenth century, a French peasant named Joseph Talon devised a novel method to enhance truffle production and cultivation. By observing that *T. melanosporum* frequently grew beneath large oak trees, he experimented with establishing new truffle beds by burying acorns under the truffle trees and transplanting the resulting seedlings into new locations. After a number of years, truffles eventually would appear under some of the young trees. Many truffle plantations were started in this manner [1].

For more than 150 years, Talon's technique was the standard method for the Périgord black truffle industry, despite its flaws [8]. The transplanted seedlings often were contaminated with pests or competing with other ectomycorrhizal fungi leading to unreliable yields. By the 1970s, French and Italian scientists began experimenting with methods for inoculating plants with truffle spores and cultures under controlled greenhouse conditions. In 1978, the first truffles produced under artificially inoculated trees were collected. The capability to establish the ectomycorrhizal relationship under controlled conditions is often considered the most important scientific development in truffle cultivation. So far, such inoculation techniques have been employed successfully to produce commercial tree plantations for *T. melanosporum*, *T. borchii*, *T. aestivum*, *T. brumale*, and *T. macrosporum*. The technique has had limited success with *T. magnatum,* likely due to poor germination of its spores [8]. Nonetheless, there is reason for optimism. A recent report used DNA analysis to trace the presence of *T. magnatum* in soil and demonstrated, for the first time, that white truffles could be cultivated outside of their native range [38]. However, truffle nurseries are famously fickle with respect to yields, often resulting in highly variable production of mature truffles. It has been said that "truffle cultivation falls in a unique place between farming and wild crafting" [13]. Put another way, these aromatic fungi "can be encouraged but not coerced to fruit" [2].

In the future, the discovery that *T. melanosporum* is a heterothallic fungus may improve agronomic practices. Strains of different mating types have to be present on the roots of host plants in order for truffle fructification to occur. Spatial separation of the two mating-types limits fruit body production; consequently, it is important that host-plant inoculation techniques manage *T. melanosporum* orchards with a balanced presence of the two mating partners [39,40].

The dramatic fall in the yield of wild truffles, the unpredictability of the truffle harvest, and their high prices have attracted criminal elements. Investigative journalist Ryan Jacobs has written an entertaining summary of the shady activities surrounding the truffle market titled *The Truffle Underground: A Tale of Mystery, Mayhem, and Manipulation in the Shadowy Market of the World's Most Expensive Fungus* [41]. The book documents the fraud perpetrated by pickers, middlemen, and others participating in the truffle trade; documents the frequency with which trained truffle dogs are stolen; as well as the "seduction" of truffle flavor and the "hidden underworld" associated with this prized luxury ingredient.

Truffles as aphrodisiacs

One reason for people's fascination with truffles is that they are among the most famous of aphrodisiacs [42]. The odor of truffles has been described as "the muskiness of a rumpled bed after an afternoon of love in the tropics" [22]. Over the ages, many people have commented on the suggestive smell of fresh truffles. For example, the food writer Elizabeth Luard has written about her encounter with a freshly dug black truffle:

> I breathe deeply. The fragrance almost overpowers me, filling my nostrils and throat with a scent so exciting, so overwhelming, so astonishingly familiar that my head swims and I have to sit down on a tree-stump. August watches me. His smile is sly, knowing. 'Ah. I see you recognize it.' And I, a mother of four, twenty years married, blush like a girl… . I breathe deeply again. The words spring to mind: sweet almonds, ripe grapes, thyme, rosemary, juniper, the scent of heather roots, bonfire embers after rain [2].

Elsewhere, Luard writes that they smell "like nothing you'd wish to describe in polite company" [2].

A major review on aphrodisiacs in history [43] cites William Cullen's 1773 *Lectures on the Materia medica*, which stated that the truffle "commonly said to be possessed of aphrodisiac virtues, is perhaps the only one which has any title to them." The same article quotes the celebrated French gastronomist Brillat-Savarin in his 1825 *Physiologie du goût* (*Physiology of taste*), who also addressed the "erotic virtue" of truffles. Brillat-Savarin recounted a story told to him by a "virtuous woman" who claimed that she had to defend herself from the uncharacteristic sexual advances of an invited guest and friend of her husband's after serving him a dish of truffled chicken. According to the story, she later was perplexed that she had not rejected the man's advances more emphatically. The story was presented to demonstrate that truffles could "on certain occasions, render women more tender and men more friendly" [43]. In another translation from the French, Brillat-Savarin's conclusion is rendered: "The truffle is not a positive aphrodisiac, but it can upon occasion make women tenderer and men more apt to love" [13].

The common folklore is that women throughout history have used truffles to arouse desire in their male companions. Moreover, it is said that Napoleon conceived "his only legitimate son after devouring a truffled turkey" and that the evil Marquis de Sade purportedly used truffles to soften up his victims [22]. The alleged aphrodisiacal properties of truffles are usually attributed to their suggestive aroma. "Not to put too fine a point on it, when ripe and ready, the truffle reeks of sex. You don't get the full impact unless you're there when it's dug" [2].

The famous Périgord truffle and Piedmont truffles are not the only species said to have aphrodisiacal properties. Deer truffles are a large group of European hypogenous fungi in the genus *Elaphomyces* [44]. Although they have received less scientific attention than other major truffle genera, folkloric traditions claim that deer are likely to rut in their presence. Ramsbottom writes that it "was long sold by herbalists under the name 'Lycoperdon nuts.' It figures as 'Fungus cervinus,' 'Tubera cervina' and similar names '*sic dicta quia reperiuntur iis in locis ubi cervi libiedenem suam exercent*'" [11].

A great deal has been made of the fact that one odor component of truffles is the steroid alpha-androstanol (5α-androst-16-en-3α-ol), also found in the saliva of rutting boars, male underarm sweat, and the urine of women. The presence of this compound in truffles provides a one-dimensional explanation for the ardor with which female pigs hunt truffles, or as essayist Nicole Walker [45] wrote aphoristically: *5α-androst-16-en-3α-ol* means "I love you in pig language." It is hypothesized that this musk odor compound is not only responsible for its attractiveness to pigs but also for its aphrodisiac-like attributes for humans [6,46]. According to Patel [25], the steroidal compound has been recognized as a pheromone that could increase the sexual arousal of women, adjust moods as submissive rather than aggressive during the female menstrual cycle, and combat anxiety and convulsion by positively modulating GABA receptors. The effects of dopamine and serotonin neurotransmitters have been postulated as leading to higher sexual arousal [47]. Dopamine can also improve genital reflexes and copulation instincts, while androstanol can activate parts of the female hypothalamus that mediate pheromone-triggered mating behavior [6,48].

Nevertheless, scientists who study smells have pointed out that "olfaction has a powerfully affective dimension. Smells can influence our mood and evoke physiological reactions as well as emotional reactions; some olfactory experiences create strong bonds with distinct memories" [49]. Like Brillat-Savarin, we agree that truffles are an expensive luxury product with such a long hold on human imagination that they can generate amatory thoughts by mere power of association.

Gastronomy

Today the most studied edible truffle species are four black truffles: *T. melanosporum*, *T. brumale*, *T. aestivum*, and *T. indicum*; two white truffles *T. magnatum*, and *T. borchii*, as well as two desert truffles, one white (*Tirmania nivea*) and one black (*Terfezia*) [3]. Descriptions of truffle aroma vary from earthy and musky to pungent and nutty; however, the desirable flavor profiles are not evenly shared amongst truffle species [3]. The Italian white truffle *T. magnatum* from South Piedmont (also known as *tartufo bianco di Alba* – Alba's white truffle) is one of the most expensive and prized food products in the gastronomic world [50].

Known to add an air of gastronomic delight, truffles are served in a variety of styles and food pairings. Many culinary connoisseurs enjoy the mushroom-like, umami flavor of truffle. In France, black truffles are often paired with foie gras as a luxury food item. Due to their pungent aroma and high price, they are often used in small quantities as a spice or flavoring in dishes, shaved into paper-thin slivers inserted into meats, or sprinkled as a garnish [1,51]. The white truffle variety is often served uncooked in dishes that include pasta, pizza, risotto, omelets, and salads [25,52]. Some Italian specialty cheeses like Boschetto al Tartufo, Sottocenere, Caciotta, and Pecorino contain truffles [25]. The famous composer, Gioacchino Rossini, called the white Italian truffle "the Mozart of the mushrooms" [8]. Here is how Ryan Jacobs, who had immersed himself in learning about the dirty underside of the truffle trade, described a dish of freshly shaven white truffle served over an al dente pasta:

> She shaved and shaved and shaved, until the light yellow of the pasta was barely visible past several layers of thin light brown slivers … Each bite, with a perfect balance of texture and salt, produced unspeakable pleasure: the form that comes with measure of silence and involuntary purrs.
>
> It was the kind of culinary pleasure that rips you away from concerns and anxieties. It made me forget where I was. It made me forget the violence, the personal betrayal, the fraud, the dog poisonings, and the theft that the truffle could have played as an accomplice on the way to my plate. It made me forget why I even cared. I could say I consciously looked the other way, but the truth is, the beauty of that moment, of that head space, was that I hadn't remembered to look at all [41].

In addition to being a premier food item in European countries like France and Italy, local truffle varieties in other countries across the world have been collected and harvested as an income source for many indigenous tribal communities, including Bedouin families in the desert where they have formed an important part of the diet [25]. Moreover, throughout the American Pacific Northwest region, the Karuk, Yurok, and Hupa people have harvested truffles since ancient times. The Igbo and Esan people of Nigeria and Cameroon also have an active, seasonal truffle trade [25]. For the desert truffles of North Africa, a common way of cooking them involves boiling the cleaned, sliced truffles in salts and seasonings, then deep-frying them in lamb oil and spices [25]. Bahrainis like to serve sliced truffles with rice or vegetables [25]; while the Kuwaiti people enjoy boiling truffles in cow or camel yoghurt or roasting them in melted butter [25]. The Bedouins, who have traditionally used truffles in ethnomedicine, eat them roasted, in soup and often substitute truffles for meat [25].

Nowadays, truffle oil is sold in many gourmet stores. It usually consists of olive oil that has been artificially flavored using a synthetic agent, 2,4-dithiapentane (syn. bis(methylthio)methane), the dominant odor volatile in the white truffle *T. magnatum* [53,54].

In addition to the limited means of production and the labor-intensive process of truffle harvesting, perishability is another factor that contributes to the astronomical price of truffles [6]. Enzymes like phenylalanine ammonia-lyase and polyphenol oxidase catalyze melanin formation from polyphenols, thereby compromising the esthetic appeal of truffles, affecting their flavor and taste, and leading to spoilage [6,55]. Since truffles are so highly perishable, they have limited shelf life as a fresh product [25]. Common storage methods include chilling, drying, and freezing. Storage at 4 °C is the treatment that best preserves the biochemical and microbiological characteristics of fresh truffles [56]. Mature truffles contain high quantities of carbohydrates and melanin (30% and 15% by dry weight, respectively) in

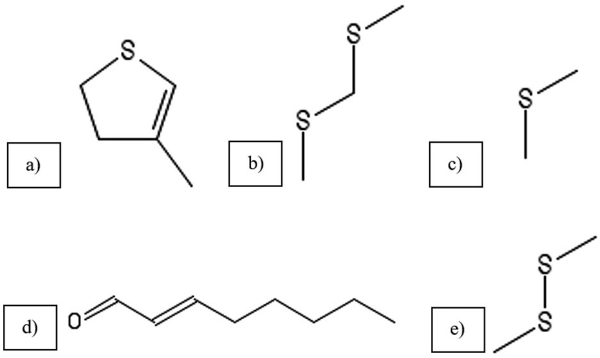

Figure 1. Major truffle compounds. (a) 3-methyl-4,5-dihydrothiphene; (b) bis(methylthio) methane; (c) dimethyl sulfide; (d) 2-octenal; (e) dimethyl disulfide.

addition to rhamnose, calcium, and iron. These compounds could potentially be used as biochemical markers to determine the degree of ascocarp development and level of maturity for quality control purposes [25,57].

Truffle aromas

The special renown of truffles, whether as luxury foods or as purported aphrodisiacs, rests upon their singular odors. The odors are caused by a blend of volatile molecules that are "shaped and presented as simplified conscious perceptions by the actively editing, synthesizing brain" [58]. Although we use a singular word, such as "odor" or "aroma" to describe scents that are as varied as raspberries and diesel fuel, almost all well-known odors are caused by a complex mixture of volatile organic compounds (VOCs). Thus, the prized truffle smell is due to a combination of individual odor molecules emitted by the fungus that vary with species, climate, weather, age, and other factors. *The Field Guide to the World's Smells* describes the component smells of the black Périgord truffle (*T. melano*sporum) as "sulfur, buttery, green apple, cheese, leather, animals, gasoline, caramel, and mushroomy." The white Alba truffle (*T. magnatum*) is said to have component smells of "garlic, onion, horseradish, cabbage, malty, mushroomy" [57]. Another source says that black truffles (*T. melanosporum*) have a "wet forest" aroma with a slight taste of radish and a tint of hazelnut, whereas white truffles (*T. magnatum*) emit a garlicky cheese aroma with subtle methane overtones [3]. Xiao et al. [59] write that truffle odor can range from mild to intense and can vary from cheese-like, earthy, garlicky, pungent, vanilla-like, creamy, leathery, and dusty, to gasoline-like. Culleré et al. [60] describe the diverse aroma of truffles as ranging from sulfur, onion, meaty, fruity green apple, anise, phenolic, metallic, mushroom, and roses to animal, butter, fruity, fatty, waxy, deep-fried, rotten food, cotton candy, and cooked potatoes. It has been theorized that the different aromatic perfumes of truffles are due to the climate and soil from which they are grown, similarly to how the terroir of vineyards confers sensory characteristics to wine grapes [6,61]. Thus, the aroma of truffles is both complex and variable. While most humans detect these VOC bouquets as attractive, different words and associations are used to convey the unique perception of truffles.

Scientists have identified more than 300 different VOCs from various truffle species, of which many are hydrocarbons with high vapor pressure and include alcohols, aldehydes, and ketones. However, the most distinctive truffle VOCs contain sulfur atoms [62,63]. Many of these same gas-phase molecules are known to be used as biochemical signals in interspecific cellular communication [64]. Unlike most fungi that spread their spores through the air, truffles are found underground and require animals to help with their dispersal [65]. It is believed that truffle odor evolved because volatiles can diffuse through the soil and attracts animals to eat and further disseminate their spores. This production of pungent cocktails consisting of volatile compounds draws a set of small animals that truffles have co-evolved with, or at least adapted to, in order to facilitate spore dispersal. At different stages of their life cycle, truffles release bouquets of specific volatiles, many of which play physiological roles in interacting with particular organisms [60,66,67]. For example, in a study of rodents that eat *Elaphomyces* species, it was found that the rodents selectively ate the truffles fruiting deeper in the soil. These species had distinct VOC profiles and produced significantly higher quantities of odiferous compounds [68]. Food and travel writer William Black stated it this way: "Truffles smell for a reason" [2].

To identify the key components responsible for the unique scent profiles of truffles, research has been done to extract and quantify their aromatic elements. Of the 30–60 volatiles characteristically produced by a single truffle species, only a small fraction contributes to the aroma perceived by humans [62]. Sulfur-containing compounds are the major contributors to the human-sensed odor of the fungus [6,62]. For example, one of the major volatile components in the white truffle, *T. borchii*, is 3-methyl-4,5-dihydrothiophene [6,62] (Figure 1). The aldehyde, 2-octenal, has been found in both white and black truffles, while bis(methylthio) methane has been determined to be the major aroma compound in the white truffle *T. magnatum* [6,64]. Several other aroma compounds have been identified in the black truffle *T. melanosporum*, including 2,3-butanedione (Diacetyl), dimethyl disulfide (DMDS), ethyl butyrate, dimethyl sulfide (DMS), 3-

methyl-1-butanol, and 3-ethyl-5-methylphenol [6,69].

Upon using solid-phase microextraction gas chromatography (SPME) to analyze nine *Tuber* species, the most abundant volatiles identified were DMS, 2- and 3-methylbutanal, 2-methylpropanol, and butanone [66]. However, the strong sulfurous component of truffles disappears rapidly when samples are left open to the air; remaining odor compounds characteristic of mushrooms like 1-octen-3-ol become more prevalent over time [70]. Truffles are highly perishable; therefore, temperature, time, and other storage parameters are primary factors influencing truffle aroma [63]. It is apparent that the main differences detected by different studies are due to regional and environmental differences in the truffles themselves, the freshness of the samples, and the analytical techniques used to assay the VOCs [71]. A useful summary of the individual VOCs detected from different truffles is available in a 2020 review by Mustafa et al. [63].

Contemporary advances in microbiome research have had an unexpected impact on the study of truffle VOCs. Recent discoveries found that many of the most characteristic truffle odors are not biosynthesized by the truffle alone, but that bacteria constituting the truffle microbiome are essential in transforming a precursor of unknown origin into thiophene derivatives characteristic of *T. borchii* aroma [72]. Furthermore, a meta-analysis of truffle aroma compounds suggests that bacteria and yeasts in the truffle microbiome play a central role forming many of the characteristic compounds associated with truffles [73].

Truffle volatiles also can be perceived by plants, and the ecological roles of these volatiles in truffle–plant interactions are an active area of research. Splivallo et al. [67] discussed the phytotoxic activity of fruiting body volatiles, and Menotta et al. [74] highlighted their potential role as mycorrhization signals. Long ago, truffle hunters observed that vegetative growth is suppressed under trees known to have subterranean truffles and it has been hypothesized that the truffles were limiting plant growth through their volatile emissions. When chemical standards of 1-octen-3-ol in the laboratory interacted with *Arabidopsis thaliana*, it was determined that this eight carbon VOC inhibited root growth and lowered the amount of chlorophyll in leaves [34,67]. Using a closed chamber assay, the volatiles released by truffles were tested on *Arabidopsis thaliana*. *T. melanosporum* and *T. borchii* fruiting bodies produced volatiles that inhibited *A. thaliana* in terms of root length and cotyledon leaf size. In some cases, the seedlings were bleached, indicating toxicity. Pacioni [75] tested the effect of ten VOCs characteristic of *T. melanosporum* and reported a significant root shortening of wheat induced by 2-methylbutanol, 3-methylbutanol, and 3-methylbutanal at a concentration of 7.5 ppm of each single VOC. Similarly, lentil roots were significantly reduced at 5.0 ppm for 3-methylbutanol, 7.5 ppm for 3-methylbutanal, and 10.0 ppm for 2-methylbutanol. Since not all truffles give rise to *terre brûlé*, it is likely that either truffle species vary in their capacity to produce these hormones or other phytotoxic compounds are produced by the species that form this barren ground [76].

Truffles as adaptogens

The US Food and Drug Administration (FDA) defines *adaptogens* as a class of metabolic regulators that have been proven to help in environmental adaptation and prevent external harms [77]. The term is mainly used to describe plant-based natural products such as ginseng that reduce a broad range of stresses [78]. However, it also encompasses extracts from several fungi that have shown benefits in traditional medicine, such as reishi and chaga [79,80].

Societies with well-developed written records reflect evidence of widespread usage of macrofungi as foods and their utility in traditional medicine [81]. For example, Tietel and Masaphy [82] have published an excellent summary of the medicinal and functional food properties of the morels (genus *Morchella*), close taxonomic relatives of truffles. Further, there is growing awareness that the nutritional value of macrofungi, including truffles, has been underrated [21,83]. Truffles contain protein, fat, dietary fiber, ash, essential amino acids, and metals (K, P, Fe, Cu, Zn, and Mn). In addition, truffles are rich in antioxidants, such as anthocyanins, ß-carotene, Vitamin C, and phenolic compounds [25], as well as ergosteroids that can be converted to Vitamin D when consumed by humans [6,84]. The chemical analysis of truffles has shown that different species make metabolites with anti-carcinogenic, anti-depressant, anti-inflammation, antioxidant, antimicrobial, immunomodulatory, and menstruation regulation properties [3,6]. For example, *T. melanosporum* contains anandamide, a fatty acid neurotransmitter that is an immunomodulator of the central nervous system and a major metabolic enzyme of the endocannabinoid system [6,85]. Truffles also produce L-tyrosine, a precursor compound for the catecholamine neurotransmitters dopamine and norepinephrine, which may confer anti-depressant activity. Additionally, Chinese truffles have potent antioxidant activity and anti-inflammatory effects [86]. In summary, truffles have been used as medicinal foods in Greek, North African, and Middle Eastern civilizations. Desert truffles have long been prized for their therapeutic properties, and several Asian species have been used in Traditional Chinese Medicine. Truffles contain certain metabolic and nutritional constituents that make them suitable as adaptogens and functional foods. Their potential as producers of bioactive metabolites with health benefits may encourage pharmaceutical companies to invest in truffle research. Moreover, it is hoped that advances in cultivation practices will make these fungi more economically accessible to the general population.

Conclusions

Truffles are economically valuable "gems of nature." Several species are prized as luxury food items, and they have a long reputation as alleged aphrodisiacs. The volatile compounds emitted by truffle diffuse in the soil and mediate interactions with microorganisms and plant roots, potentially regulating a complex molecular dialogue among soil, fauna, vegetation, and microbial life. Truffle formation is a complex process that has attracted the attention of naturalists for centuries. These subterranean fungi do not form fruiting bodies unless they enter a symbiosis with plant roots and establish ectomycorrhizae. In nature, truffles depend on animals to spread their spores and emit potent volatiles to attract animal vectors. These same volatiles contribute to their delectable aroma and their importance in gastronomy. There has been recent success with the cultivation of black truffles, and the controlled production of white truffles is showing promise. Some truffle species also have potential as medicinal agents or adaptogens. As their culinary popularity continues to grow, and as pharmacologists discover that they have potential clinical uses, mycologists hope to identify new, delectable species, test new host plants for cultivation, and discover means for greater production to increase the supply of truffles throughout the world.

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