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Food, Health and Agricultural Importance of Truffles: A Review of Current Scientific Literature

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Abstract

Truffles, the subterranean mycelial fruit bodies are wonderful gustatory delights across the globe. Of late, several therapeutic compounds with antioxidant, immunosuppressor, antimicrobial, and anticarcinogenic properties have been discovered from truffles. The so far unexploited fungal resource truffles have been recently discovered to possess great economic potential. However, their cultivation methods have not proved much successful. Submerged fermentation for higher production yield, use of response surface methods for optimum polysaccharide extraction, innovative storage techniques to eradicate spoilage, adulterant determination in canned truffles by molecular tools are strategies for their optimal utilization. In the present paper, the issues hindering the popularity of truffles, the current scenario and the future potentials are reviewed.

Key words: Truffles, antioxidant, antimicrobial, anticancer, mycorrhiza, fermentation

Introduction

The etymological origin of truffle is from *tuber*, meaning "lump". The Latins refer to it as Tuber, derived from the word tumere (to swell) to indicate its globoid form. In scientific jargon, truffles are microaerobic, hypogeous, ascomycetous fungi that form ectomycorrhizae (ECM) with the roots of both angiosperms and gymnosperms. Generally, truffles form long-living symbioses with oak, elm, poplar, chestnut, willow, hazel, beech, birch, hemlock, fir and pine (1). Since antiquity truffles are regarded as the ultimate gastronomic delight or "the diamond of the kitchen"(2). This group of fungi generally belong to the family Tuberaceae or Pezizaceae (3).

Despite their multiple nutritional and therapeutic importance, truffles are shrouded with mystery since antiquity. The scripts on papyrus document that, truffles have been relished by the rich and famous since the Pharaoh age. The truffles are thought to be a "miracle of nature" since ancient Greek civilization. Theophrastus, a pupil of Aristotle's, referred to truffles in 500 BC as "a natural phenomenon of great complexity, one of the strangest plants, without root, stem, fiber, branch, bud, leaf or flower." The precious desert truffle of the Middle East is believed to spawn when lightning and thunder strikes. Since, 18th-century, edible truffles are held in high esteem in French, Spanish, northern Italian, Croatian and many other international cuisines. Truffles are considered to be one of the oldest food-stuff used by Arabs. The Bedouins used truffles as a substitute for meat in their diet (4).

Desert truffles are edible, seasonal and socio-economically important fungi, growing wild in the central-southern part of Bahrain. The truffles usually appear in the deserts of Gulf States following the rainy season (5). Demand of truffle exceeds supply because only ~20 tons are produced worldwide, so price even reaches to an exorbitant US$3000 per kg for white truffles. To cater to the overwhelming consumer demand, large plantations have been established in southern European countries, New Zealand, Australia and the USA, for harvesting truffles.

***Morphology, types and distribution*:** Freshly dug up truffles are aromatic, wrinkled and have bruised, lobed potato-like appearance. Tuber species can generally be distinguished on the basis of their fruit bodies and mycorrhizae (6). The white species are *viz. Tuber magnatum*, *T. maculatum*, *T. borchii*, *T. dryophilum*, *T. puberulum* and the black are *T. brumale*, *T. melanosporum*, *T. indicum*, *T. himalayense*. *T. magnatum* is the most hunted and prized truffles species (7) (Fig 1). The flavour of black truffles is far less pungent and reminiscent of fresh earth and mushrooms.

It is generally assumed that truffles species diversity is favoured by warm, fairly dry climates and calcareous soils. There are about 100 different kinds of truffles around the world, most of which grow in various parts of Europe, particularly in France, Italy, Australia, China, deserts (3). In Piedmont, Tuscany, Umbria, and Le Marche regions of Italy, truffles are found in plentiful during October and November. Arid desert areas harbour truffles, notably *Terfezia* and *Tirmania* species belonging to Pezizaceae family (8). *Tirmania nivea* or "Zubaidi" is the most preferred and expensive truffles in Baharin due to its musky smell, delicacy and soft white tissues. This is followed by *Terfezia claveryi* or "Ikhlasi". *Terfezia boudieri* chatin is widely distributed in arid and semi-arid regions of Tunisia. In the Pacific coast of North America, includng Oregon and California, white truffles *(Tuber oregonense)* and *Tuber gibbosum* are harvested.

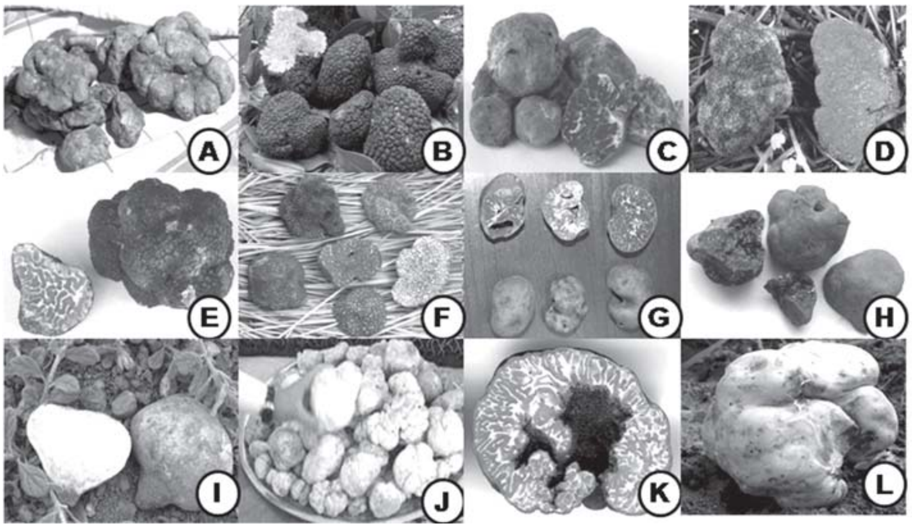

Fig. 1. A. *Tuber magnatum*, **B.** *Tuber aestivum*, **C.** *Tuber borchii*, **D**. *Tuber melanosporum*, **E.** *Tuber brumale,* **F.** *Tuber indicum*, **G.** *Tuber excavatum*, **H.** *Melanogaster intermedius,* **I.** *Terfezia claveryi*, **J.** *Tirmania nivea,* **K.** *Tuber pseudoexcavatum,* **L.** *Choiromyces meandriformis Vitt.* (Collected from www.wikipedia.org/)

***Biochemical composition*:** The chemical composition and nutritional quality of *T. claveryi* and *T. nivea* have been studied, which show the presence of protein, fat, dietary fibre, ash, ascorbic acid and essential amino acids, K, P, Fe, Cu, Zn and Mn (9). Recent studies have proven that some truffles contain ergosteroids, ergosterol, the most widespread fungal sterol and brassicasterol having the characteristic sulphurous aroma (3, 10). The typical taste of truffles is the result of a unique combination of several volatile organic compounds (VOCs) *viz.* aldehydes, alcohols, ketones, organic acids and sulphurous compounds (11). The aroma of truffles are diverse, ranging from sulphur, onion, meaty, fruity green apple, anise, cheese, phenolic, metallic, mushroom, roses, earthy, dust, gasoline, leather to animal, butter, creamy, fruity, fatty, waxy, deep-fried, rotten food, cotton candy and cooked potatoes (12). The most important aroma compounds of black truffles are 2, 3-butanedione, dimethyl disulphide (DMDS), ethyl butyrate, dimethyl sulphide (DMS), 3-methyl-1-butanol and 3-ethyl-5-methylphenol (12) (Fig 2). The polyhydroxylated ergosterol glycoside, the dominant unsaturated fatty acids as well as a minor polyhydroxylated C18 fatty acid have been discovered from the fruiting bodies of *T. indicum* (13). Quinonoid and polyphenolic compounds are the most important constituents of truffles pigments. The black pigments are found to be allomelanins of polyketide origin (14). Truffles VOCs derived from various metabolic pathways *viz.* fatty acid catabolism, polyketidic and isoprenoid pathways, are small hydrocarbons containing alcohol, ester, ketone, aromatic groups and sulfur atoms (15). The factors characterizing the mature truffles are a relatively high level of carbohydrates and melanin (30% and 15% by dry weight, respectively) and the presence of rhamnose, calcium and iron. These biochemical markers could be used as indicators of the degree of ascocarp development and the attainment of maturity (16).

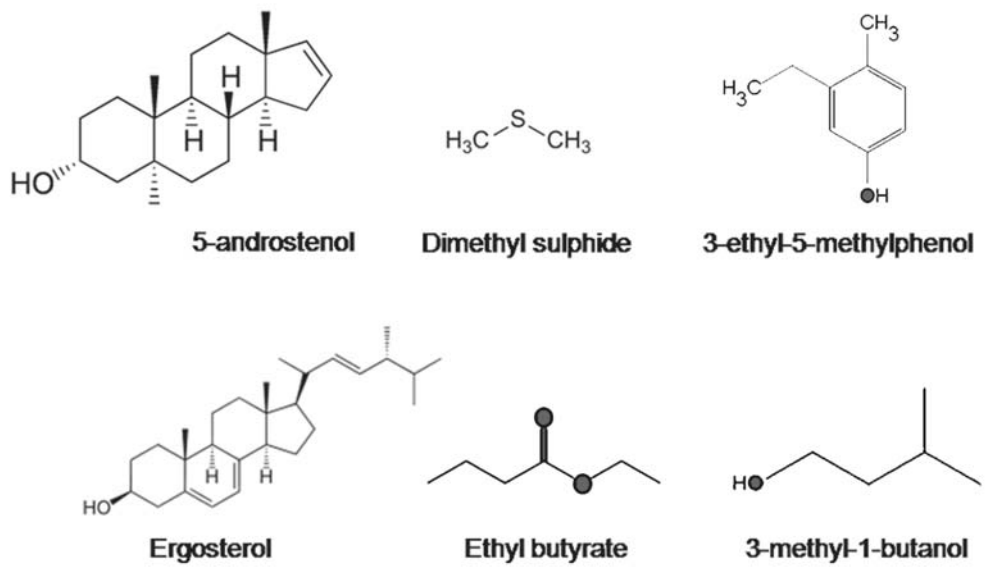

Fig. 2. Various active compounds of truffles *viz.* 5-androstenol, dimethul sulphide, 3-ethyl 5-methylphenol, ergosterol, ethyl butyrate and 3-methyl 1-butanol.

***Structure elucidation of bioactive compounds*:** Bioactive compounds are extracted from dried and powdered fruiting bodies using $CHCl_3$ and methanol, followed by silica gel column chromatography (17). In order to determine the alkyl chain length in fatty acids and sphingosine components, the acid hydrolysis is carried out (3). A headspace solid-phase micro-extraction combined to gas chromatography mass-spectrometry (GC/MS) has permitted the aroma identification of diverse truffles species. (18). A novel method using solid-phase extraction coupled with gas chromatography (GC) and flame ionization detector for the quantitative determination of 5-androstenol in truffles fermentation broth is developed (19). The methods of head-space and vapour analysis at high mass resolution have permitted the identification of 36 compounds in the volatile fractions from 6 species of truffles collected from France. The stir bar sorptive extraction in head space mode coupled with GC/MS has been successfully applied to compare the aroma profile of three truffles species (15). The aromatic composition is evaluated by gas chromatography-olfactometry, complemented by an aroma extract dilution analysis (12).

***Multiple uses of truffles*:** Apart from their immense demand in gastronomic platter, the truffles have also therapeutic potential. The role of truffles in ethno-medicine of Bedouins is documented in Islamic literature (20). These fungal wonders have also antioxidant, antimicrobial, antimutagenic and aphrodisiac properties. Truffles are also good source of sterols. Truffles also can supplement the meagre income of the tribals. Truffles play pivotal role as mycorrhiza too, suggesting their obvious contribution to agriculture.

***As food*:** The arresting aroma and delectable taste makes truffles prized in the culinary world. Connoisseurs relish truffles products like truffles oil, jams or biscuits (20). Truffles, generally the white varieties can be served uncooked as pasta, pizza, omelette and salads (2).Truffles can also be cooked in a myriad ways. The most favoured way of cooking truffles include boiling the cleaned, sliced truffles in salts and spices followed by deep frying in local lamb oil and spicing. Bahrainis prepare sliced clean truffles with rice or other vegetables. Kuwaiti truffles prefer to boil truffles in salty, fresh cow or camel yoghurt or roast them in melted butter, while other Bedouins eat them roasted or as soup. Roasting in campfore ashes is another method of cooking. Sometimes the boiled trufflesare added to a sauteed onion, garlic, and tomato sauce flavoured with spices. White or black paper-thin truffle slices may be inserted into meats or sprinkled as garnish. Some speciality cheeses contain truffles as well. A savoury Tuscan cheese, Boschetto al Tartufo is made with cow and sheep milk infused with thin slices of local truffles. Sottocenere, Caciotta and Pecorino are some other popular Italian truffle speckled-cheeses. Bedouins, usually use truffles as a substitute for meat. Truffles are also used to prepare truffle vodka. Truffles can be kept for future use by drying. The 'desert truffles' are of high dietary value. The most valued truffles in Iraq cookery is the *Terfizia* species for its delicious taste. *T. nivea* can convert a bland recipe into a delicacy (5). Truffles are one of the most expensive food articles in Europe, especially in Italy and France which are mainly relished uncooked (21, 12). *Tartufo Bianco,* a risotto made with white truffle is a delicious Italian special dish. The nutritional value of truffles are higher than that of the cultivated mushrooms, largely owing to lower water contents. The sweet taste is because of the high carbohydrate content. Peeling of truffles before eating has been reported to considerably decrease the content of protein, fat, ash, ascorbic acid and mineral elements especially calcium and iron. While in the past, chefs used to peel truffles, in modern times most restaurants brush the truffle carefully and shave it or dice it with the skin on so as to retain the nutrients as well as to use most of this expensive stuff. Supplies can be found commercially as fresh produce or preserved, typically in brine, as canned truffles.

***As antioxidants*:** Truffles have high content of antioxidants such as vitamin A, C, β -carotene and phenolic compounds, which can scavenge peroxy radicals and chelate ferric ions, thus reducing lipid peroxidation (5). The effect of industrial processing as canning and freezing on antioxidant activity of edible truffles *T. claveryi* and *Picoa juniper* were studied (15). The antioxidants in dried desert truffles from Bahraini, Iranian, Moroccan and Saudi origins have been examined for their antiradical activities by ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), deoxyribose, and nitric oxide (NO) methods (5). Chemical constituents responsible for the antioxidant activities have been identified as ascorbic acid, anthocyanins, total esterified phenolics, total free phenolics and total flavonoids and total carotenoids. The antioxidant rich aqueous extract of *T. claveryi* have demonstrated a very powerful hepatoprotective activity, when evaluated in rats using a potent hepatotoxin carbon tetrachloride (CCl_4) (20). Freezing and canning decreased the antioxidant activity of truffles (22).

***Antimicrobial property*:** Recently reported antibiotics extracted from the desert truffles *T. nivea* and *T. claveryi* have proved effective against a broad range of Gram (-) and Gram (+) bacteria (20, 23). *T.claveryi* was reported to be useful in the treatment of opthalmic ailments. Boiled water extract of truffle was claimed effective by the Bedouins for trachoma remedy. The extract showed inhibition towards the aetiological agent of trachoma, *Chlamydia trachomatis* (20). The antimicrobial activity of aqueous and methanolic extracts, as well as partially purified protein extracts from *T.claveryi* was investigated against *Staphylococcus aureus (*24*)* and *P. aeruginosa* (25) *in vitro*. Aqueous extract of *T.claveryi* was reported to contain a potent proteinaceous antimicrobial agent for treatment of eye infections caused by *P. aeruginosa*.

***Immunomodulating, antitumor and antimutagenic property*:** Possible mutagenic and antimutagenic properties of aqueous and ethanolic extracts from *T. aestivum* have been studied (11). Intracellular polysaccharides (IPS) isolated from the fruiting-body of *T. sinense* have demonstrated immunomodulatory and antitumor activities (26). Neurotrophic, antitumor, immunostimulatory, anti-phospholipase A2 and cholesteryl ester transfer protein inhibitory activity have also been reported from some truffles (3).

***As aphrodisiac, anti-depressant and sterol source*:** For centuries, truffles have been believed to possess mystical aphrodisiac powers. Food connoisseurs describe the scent of truffles as sensual and seductive. A survey has reported that, about 95% of the non-Bahraini respondents and 72% of the Bahraini respondents eat truffles for sexual reasons (20). 5α-Androst-16-en-3-α-ol (androstenol), a steroidal compound belonging to the group of musk odorous 16-androstenes, recognized as a pheromone that could increase the sexual arousal of human female, adjust moods as submissive rather than aggressive in female menstrual cycle and antagonize anxiety and convulsion by positively modulating the GABA receptors. Due to its pleasant odour and pharmaceutical function, androstenol has been developed to perfume with high value in market.

Also, it has potential in drug development for anti-depression (19). From the fruiting bodies of *T. indicum*, a new polyhydroxy sterol glycoside named tuberoside has been isolated along with four known ergostanes. These compounds are assumed as structural constituents of cellular biomembranes and precursors of steroid hormones (17).

***As model for investigation of enzymatic adaptation*:** Truffles show both morphological, physiological and biochemical adaptations to poorly oxygenated area and are good models for investigation of enzymatic adaptation to microaerobic conditions. The antioxidant and glutathione dependent enzymes of truffles *viz.* superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glyoxalase-1 and glyoxalase-2 are expressed and correlated with the microaerobic metabolism, growth rate and mycorrhizal symbiosis of truffles (1).

***As source of income*:** Many indigenous tribal communities are dependent upon truffles not only as diet but as earning source. Bedouin families in the deserts are motivated to supplement their income by collecting truffles (20). The Karuk, Yurok and Hupa people of Pacific Northwest region practise truffle harvesting since ancient times. For the Igbo and Esan people of Nigeria and Cameroon, mushroom hunting is dominated by the women, who gather and sell truffles for seasonal income. While *T. melanosporum* is generally sold at euro 30-40/100g in France, *T. magnatum* reached euro 300–400/100g in fall 2003. A productive truffle-ground represents therefore a significant economic source.

***As mycorrhiza and nitrogen fixing agent*:** Ectomycorrhizal fungi have received a great deal of attention in recent years and have been used world-wide in reforestation, since they can ramify their hyphae in soil and enhance water and mineral absorption of host trees and provide protection against root diseases. In this regard, truffles are important. Morphological characterization of mycorrhiza formed by *Helianthemum almeriense* with *T. claveryi* and *Picoa lefebvrei* have been studied (27). Ectomycorrhizal fungi are also evidenced to confer their host plants with increased tolerance toward toxic metals. Metal homeostasis-related genes in *T. melanosporum* have been recently identified. Genes associated with metal transport exhibited preferentially highest expression level in mycorrhiza, suggesting extensive trafficking of metals as Cu and Zn to host root cells (28). Diversity of nitrogen-fixing bacteria and their activity was investigated in *T. magnatum*, the most well-known prized species of Italy. Degenerate PCR primers are applied to amplify the nitrogenase gene *nifH* from *T. magnatum* ascomata which revealed the presence of *Alphaproteobacteria* belonging to *Bradyrhizobium* spp. (29).

***Searching and detection*:** The high cost of truffles, arise from their difficult and labour-intensive treasure-hunting dedication. Pigs can detect truffles underground owing to aromatic pheromones emanating from both black and white truffles (3). The female pig's instinct to comb the forest floor for truffle is due to a compound within the truffle similar to androstenol, the sex pheromone. In Italy and France, small groups of truffle hunters scour the woods with dogs and pigs looking for truffles in secret spots. Trained pigs and dogs through their ability to detect and recognize the odorant VOCs, determine the underground locations of truffles. While pigs have the keener nose for truffles, they tend to eat the truffles, so dogs are preferred as they have little appetite for mushrooms. Dimethyl sulfide appears to be the key-odour compound for truffle location. Two other sulfurous and three C8 compounds are reported to be attractant for truffle flies (2). The locations of truffles can be detected by observing the hovering of *Suillia* flies as they lay eggs on the ground above truffles, to provide food for the larvae. Knowledge on the microbial environment where truffles develop is surely a pre-requisite for a better exploitation of the natural truffle-grounds (30). Truffle hunters are a secretive breed who rarely part with the tricks of their trade, do not encourage outsiders into their fraternity and fight all attempts to regulate or organise the truffle business and this craft is usually handed down from generation to generation. Truffles are hunted in the Kalahari Desert by men and women who look for cracks or humps in the soil, which are then extracted with hands or digging sticks.

***Dispersal and cultivation*:** Squirrels, wallabies, mice and voles browse on truffles and play an essential role in spore distribution. Mycologists have tried to cultivate truffles by injecting the spores into the roots of trees. This process has met with some success in the case of the black truffle; however, it has proved futile with the most expensive and prized white truffle. Irrigation enhanced the production of truffles due to the abundance of the *Helianthemum* herbs in the Iraqi, Syrian, Jordanian and Saudi desert (21). Several statistical studies have indicated that a high concentration of active carbonate in the soil favours *T. melanosporum* fruit body production. It is explained as the acidification of the immediate soil environment and solubilisation of carbonated fractions by *T. melanosporum* mycelia favouring their mycelia growth (31). *T. melanosporum* has ability to create vegetation-free area because its mycelium and fruit bodies produce multiple substances that adversely affect young plants and seed germination, such as 2-methylpropanal, 2-methylbutanal and 2-methyl-1-butanol (31).

***Hurdles in popularity*:** Because of their elusiveness and high price, truffles are used sparingly. Only the hunters or the rich have access to the taste the delectable truffle dishes, common people are often deprived. Limited shelf life of truffle as fresh product is another deterrent, as storage robs off its taste, aroma and hence marketability. This problem is particularly prevalent in Italy, where production has high potentialities, but low possibilities for its wide marketing, due to its limited shelf life as a fresh product. The mating system and the mode of spore dispersal are still under shroud, although some mammals or insects are suspected to act as potential vectors. This general lack of ecological knowledge account for the difficulty in artificial inoculation of host trees with truffle spores (32).

***Spoilage*:** Stress factors cause release of enzymes phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) in truffles. PAL is involved in polyphenol synthesis, whereas PPO is responsible for catalyzing the biochemical conversion of the phenolic compounds. PPO oxidizes polyphenols to quinones, which form brown melanin pigments (23). Enzymatic browning affects the flavour and taste of truffles. Perishability of truffles is accelerated when stored in humid places. Fresh truffles are strongly contaminated from different groups of microorganism *viz. Pseudomonas*, *Listeria*, *Salmonella*, mold and yeast species (33).

***Strategies for enhanced production*:** Because of the great demand for truffle in market and the shortage in wild resources, an innovative strategy for truffle production is urgent. Comparing to the natural fleld collection and semi-artificial simulation cultivation, submerged fermentation is a promising alternative for the efficient production of truffle and its metabolites. A fed-batch mode of submerged fermentation process is developed for the efficient production of bioactive mycelia and polysaccharides from *T.*

melanosporum. The production of exopolysaccharides (EPS) and intrapolysaccharides (IPS) are markedly improved by carbon and nitrogen sources mixing feeding strategy (34). Another novel method using Plackett-Burman design (PBD) coupled with Draper-Lin small composite design (SCD) and desirability function (DF) was developed to optimize *Tuber melanosporum* fermentation medium. Compared with the original medium, the biomass, production of EPS and IPS were increased upto 54%, 72% and 124%, respectively (35). Effects of nitrogen source and its concentration were studied during the submerged fermentation of *T. sinense*. A mathematical model constructed by Box-Behnken design and response surface methodology was applied to study the synergic effect of carbon and nitrogen sources which identiûed yeast extract and peptone as the most favourable ingredients for mycelial growth (36). For large-scale fermentation of truffles for simultaneous production of biomass and tuber polysaccharides, fermentation technique proved promising.

***Storage and sterilization*:** As already mentioned above, the truffles are highly perishable. These are incapable to retain their sensory and biochemical peculiarities for a long time. The common methods used to store truffles include chilling, drying and freezing. Some Bedouins preserve clean truffles by pickling in 3-6% vinegar and salt (20). Use of gamma rays, are potentially attractive to improve the shelf life and safeguard sensory characteristics of truffles. Effect of 1.5 kGy gamma-ray dose on some biochemical and microbiological profiles of black truffles were monitored, immediately after treatment and after 30 days of storage at 4°C and found to be suitable for preservation (23). However, if applied in an inappropriate mode, irradiation could trigger unwanted sensory and chemical changes, i.e. resulting in free radicals, whose reaction with proteins, lipids and polyphenols, could give rise to detrimental effects. The effectiveness of radiation, heat and fungicides separately or synergistically to inactivate the fungal flora present on truffles were investigated. A trial of triple combination of 2000 ppm propionic acid at 56°C for 5 min and 150 krad of ionizing radiation brought complete sterilization against microbial spoilage (21). Electrophoretic and chromatographic analyses of proteins and peptides allowed a better understanding of the mechanisms responsible for biochemical alterations and bacterial pattern in black truffles during their storage. The γ– irradiation at 1.5 kGy appeared as threshold dose for preserving the characteristics of the fresh product beyond which polyphenol degradation is observed (37). Recent progress in food technology as well as a better knowledge of food physics and chemistry, microbiology and engineering, has allowed the introduction of more innovative food storage systems (23). Storage at 4°C is the treatment that best preserves the biochemical and microbiological characteristics of fresh truffles (38). Subjecting truffles to high CO_2 and low O_2 atmospheres reduce the polyphenol metabolism, anerobic pathways and polyamine biosynthesis slowing senescence (39). Falsconi *et al.* (2005) studied the relative change of the white truffle's aroma (*Tuber magnatum* Pico) in the days following the harvesting, in order to determine the maximum preservation time for the white truffles (Alba's truffle). The flavour of the white truffle is mainly characterized by four parameters: the type, the origin, the ripening and the freshness (aging). The truffle freshness is extremely important for both consumer's safety and commercial points of view, i.e. determining the quality and price of the product. It would be therefore interesting to have a reliable system for truffle freshness evaluation. The change in aromatic compounds in the headspace of white truffles using SPME-GC-MS technique and the Pico 2-electronic nose (EN) were observed (40). A significant change in truffle's aroma has been observed after 5 days from harvesting. This variation has been mainly attributed to an increase in the headspace of four compounds: acetic acid, ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol. All these compounds are originated by truffle fermentation and can be considered as markers of the product degradation.

***Adulterant determination*:** Purveyors often cheat the consumers by selling minor league truffles at major league prices. To protect consumers from fraud, to identify less tasty and cheaper truffle species, PCR DNA-based method is helpful. This technique unequivocally identifies the nature of the product. A DNA extraction kit in association with a mitochondrial PCR marker is useful to analyze canned truffles (22).

***Molecular studies* :** Integration of molecular and geographic diversity patterns can allow the selection of sites for *Tuber* biodiversity conservation (41). Phylogenetic relationships among truffle species from Europe and China were investigated through parsimony analysis of the ITS sequences. Three major clades were obtained (42). Nuclear and mitochondrial ribosomal DNA (rDNA) polymorphisms were used to analyse the genetic variability in natural populations at different geographical scales (32). Genetic diversification of *Tuber magnatum* depending on the different environmental conditions were analysed with multilocus horizontal starch gel electrophoresis (7). Speciûc primers, based on the *T. magnatum* internal transcribed spacer (ITS) of the ribosomal DNA sequence, were used for a molecular characterization of mycorrhizal seedlings raised under controlled conditions. Morphotyping and ITS sequencing of these mycorrhizal samples provided novel information on the ectomycorrhizal and endophytic species living in a *T. magnatum* truffle-ground (30). Degenerate PCR primers were used to amplify a conserved gene portion coding chitin synthase from genomic DNA of six species of ectomycorrhizal truffles, *T. magnatum* and *T. ferrugineum*, while TubCHSZ was derived from the in vitro growing mycelium of *T. borchii* (43). The cloning, expression and characterization of the hxk-1 gene of white truffle *Tuber borchii* Vittad was studied to understand sugar metabolism (44). Degenerated oligonucleotides were used as primers for polymerase chain reactions to amplify PKC-related sequences from the white truffle species *Tuber magnatum* and *Tuber borchii*. The deduced amino acid sequences of cloned sequences reveal domains homologous to the regulatory and kinase domains of PKC-related proteins, but lack typical Ca^{+2} binding domain and therefore should be classified as nPKCs. Both contain a large extended N-terminus which is found exclusively in fungi PKCs (45). Three black truffle species were studied and found that they can unambiguously be differentiated by performing a single amplification reaction and comparing the length of amplicons obtained (46). Truffles grow in arid reasons. Owing to disturbance of the sandy soils around the Kalahari villages by cattle and goats, the truffle harvest is steadily getting endangered. Taylor *et al* 1995 worked on restoration of production and devising ways for enabling the rural poor community to cultivate truffles for food (47).

Conclusions

Truffles despite their wonderful organoleptic, pharmaceutical and agricultural possibilities have not got its due recognition, which needs to be unravelled for their maximum exploitation. To raise public awareness, truffle fairs are being held in the regions where these are harvested *viz.* Italy and Oregon. It is a matter of concern that, natural truffle production has declined dramatically over the past century, the main obstacle being the inadequate knowledge of their cultivation. However, the advent of molecular biology is expected to give a boost to truffle research. The rapid strides in food processing techniques have accorded truffles a reputed status in international food platter as gastronomic delight and potential nutraceuticals. However, further scientific studies are warranted in order to develop value-added products, functional foods and pharmaceuticals from bioactive compounds from truffles. As the popularity of truffles is growing, the developing countries are attempting to cultivate truffles for exporting, to boost their economy. Mycologists are engaged in active research identifying new truffle species, testing new hosts, inventing innovative cultivation practices for mass farming of truffles. For all the information available on truffles, it is still in infancy, still an untapped source with multi-pronged prospects. This updated review is strongly believed to provide future direction in truffle research.

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