Truffles

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Truffle is a gourmet food used in the haute cuisine world for its valued and pleasant aroma. In scientific jargon truffle is an ascomyceteous fungus belonging to the Tuberaceae family of the Pezizales order. Truffles are underground edible fungi that grow symbiotically with plant roots. They have been globally considered as one of the most expensive foods because of their rarity, unique aroma and high nutritional value as antioxidant, anti-inflammatory, antiviral, hepatoprotective, anti-mutagenic, antituberculoid immunomodulatory, antitumor, antimicrobial, and aphrodisiac. The unique flavor and fragrance of truffles is one of the main reasons to get worldwide attraction as a food product. Truffles possess significant variability in their aroma profiles from species to species. In general, sulfur compounds such as dimethyl sulfide (DMS) and dimethyl disulfide (DMDS), 1-octen-3-ol, and 2-methyl-1-propanol have been identified in most truffle species.

Keywords: truffles ; aroma ; volatile organic compounds ; analytical methods ; GC

1. Introduction

Although some fungi are able to form underground fruiting body, e.g., basidiomycetes, only those of the genus *Tuber* are considered true truffles [2]. The word *truffle* probably derived from the Latin *tubera* the plural of *tuber* that means *lump*, *hump,* or *swelling.* Latin called this fungus *tuber* that probably descends from the word *tumere* to indicate its globoid form [1]. In the *Tuber* genus have been discovered more than 200 species and the most of them remain undescribed mainly because many of the species produce small size fruiting body that are morphologically cryptic and lack of any culinary values [3]. On the other hand, some species are extensively appreciated and hunted in several countries. In Europe occur the most valuable truffles, i.e., *Tuber melanosporum* Vittad. (Périgord black truffle), *Tuber magnatum* Pico (Italian white truffle), *Tuber aestivum* Vittad. (summer or Burgundy truffle), and *Tuber borchii* Vittad. (bianchetto truffle) (Figure 1).

Figure 1. Most valuable truffles occur in Europe such as (from top to bottom) *T. melanosporum*, *T. magnatum*, *T. aestivum,* and *T. borchii*. (Photos have been provided by “Mycology Collections Portal” https://mycoportal.org/portal/collections/harvestparams.php?db%5B%5D=25&x=46&y=24, accessed on 20 November

2020).

The most important harvested species in China is *Tuber indicum* Cooke and Massee, an Asian black truffle similar to Périgord black truffle but with less flavour. *Tuber oregonense* Trappe, Bonito and P. Rawl., *Tuber gibbosum* Harkness, and *Tuber lyonii* F. K. Butters (pecan truffle) occur in America but they present only restricted market [2][4]. Other species that are popular mainly in the Middle East, Mediterranean basin, and Northern Africa are the so-called desert truffles. These ascomyceteous fungi, belonging mainly to the genera *Terfezia* and *Tirmania*, grow in arid and semiarid areas [5][6].

Truffle depends on a suitable plant host with which generates an obligate symbiosis. In fact, it forms a network composed by branched hyphae connected with the plant roots called ectomycorrhizal. This structure with large surface area allows mutually beneficial exchanges of resources between the plant host and the truffle. Without this association the fungus cannot produce the mature fruiting body and therefore complete its life cycle [7]. These fungi have evolved to attract dispersal agents such as insects and mammals by producing intense aroma [2]. This strong aroma and unique flavour are the reasons why truffles are sought-after in the global market and the inability of the production to satisfy the market demand establishes the high price of this gourmet food. Several factors affect the production and therefore the truffle price such as difficulties in truffle cultivation, season variability, manually harvesting that require trained animals and climate changes [7].

2. History

In ancient times, truffle was known and consumed by Babylonians, Etruscans, Egyptians, Greeks, and Romans. This fungus was vailed by a great mystery since it was unknown where it comes from[8]. For example, an Aristotle' disciple, Theophrastus, reported that truffle is born from autumn rains or flash of lightning. One hundred years after Theophrastus, Nicander, a Greek poet, supposed that truffles were silt modified by a source of internal heat. One century and half later, Plutarch believed that they were cooked in the mud by lightning. The truffle mystery was more emphasized by its magical fragrance and presumed aphrodisiac quality. For example, Galen prescribes it to his patients because he believed that it was very nourishing, and it generated a general agitation that conduced to sensual pleasure. During the Middle Ages truffle missed popularity even if some scripts report its usages and how to hunt it. For example, some account books proved that truffle was served during the wedding feast of Charles VI of France and Isabeau of Bavaria in 1385 [9]. At the beginning of 18th century Joseph Geoffroy, pharmacist and botanist, made some observations that have been essential to classify truffle as fungus. His observations were then confirmed by an Italian botanist, Pier Antonio Micheli, who described the truffle spores. Important researchers such as Carlo Vittadini and the Tulasne brothers, considered the founders of the modern mycology, firmly studied truffle during the 19th century [8]. In 1808, Josef Talon, a French farmer, developed the first method for truffle cultivation that consisted of planting seedlings that were collected under the oaks where truffles have been found; only thanks to Auguste Rousseau this idea was disseminated and thousands of hectares of oaks were planted in France [5][10]. The method of Talon has been used for more than 150 years until some French and Italian scientists proposed the technique of nursery seedling inoculation [8].

3. Cultivation

The first tentative of cultivation, as already mentioned, was performed by Josef Talon during the beginning of 19th century. Using the method of Talon, which consisted of planting acorns under the oaks that produced truffles and transplanting the resulting seedlings in new area, vast plantations were established in France and successively in Europe [11]. The second part of nineteen century is known as the golden age of truffle production also thank to the Talon method while a marked decline, especially in France, occurred in the first half of 20th century. This falling down of production can be probably ascribed to the two World Wars. The inoculation of seedlings in the nursey with various species of *Tuber* together with the usage of spores or segments of infected roots has been a big progress on truffle cultivation [5][12]. Various species such as *T. aestivum, T. borchii* and *T. melanosporum* are cultivated in various countries but *T. melanosporum* still remains the main farmed species in different areas of Europe, and in other non-native places such as Africa, Asia, Australia, and America. Desert truffle such as *Terfezia claveryi* Chatin is cultivated in Spain, Israel, and Abu Dhabi using as plant host *Helianthemum* species [4]. Cultivation of the most expensive truffle, the Italian white truffle *T. magnatum*, is not still as productive as that of other species despite several attempts mainly established in Italy since the early 1980s. This is likely due to some difficulties during plant inoculation with the truffle and contaminations that generally occurred [2]. In fact, some fungi can compete with truffle for capturing space on the host roots in the nursery and later in the field. Therefore, the resulting tree will not produce truffles or solely few fruiting bodies. It is important to point out that to obtain a satisfactory production of truffles is basic to use not only an inoculated plant but a well-infected seedling with only one *Tuber* species [12]. This is the reason why truffle growers have required, since several years, a unique certification protocol in Europe for evaluating and certificating the *Tuber* infected plants by molecular and morphological analysis with the aim to protect the

truffle farmer for failures [2]. The selection of host trees and diverse environmental factors play an essential role on achievements in truffle cultivation as well. The most cultivated truffle (*T. melanosporum*) has a high number of host trees belonging to different genera such as *Quercus*, *Corylus*, *Populus*, *Tilia*, *Ostrya*, *Carpinus*, *Cistus*, *Pinus,* and *Cedrus* [13]. This black truffle in Europe grows from near sea level in France up to 1800 m in Spain and there are some discrepancies among truffle growers and experts for the selection of right field altitude of cultivation. Another important parameter seems to be the soil slope since wild truffle generally grows up to 60% of the slope. Probably this condition could facilitate the water drainage. Precipitation and temperature also affect the results on truffle cultivation as well as the characteristic of the soil, e.g., stoniness, acidity and alkalinity, minerals, texture, and structure. An alkaline pH (7.5–8.5) and a granular, well aerated structure with good natural drainage soil are positive conditions for black truffle cultivation [11][14]. Further molecular studies on truffle life cycle and mycorrhizal symbiosis could allow to a more productive cultivation for example by selecting a specific mycelian inoculum adapted for the host plant and climatic and edaphic conditions[2].

4. Uses

Truffle is widely appreciated and consumed for its unique, valuable, and pleasant aroma besides it is one of the most expensive foodstuffs. In fact, it is known as “underground gold” or “diamond of the kitchen” and it is considered a costly delicacy. Although it can be alone consumed as food it is generally used as spice to enhance the dish savour as aroma flavouring [15][16]. In fact, it is added to different dishes such as meat, pizza, pasta, risotto, and eggs. For example, an Italian special dish is a risotto with white truffle. Truffle can be served raw as thin slices added directly to the dish and or sprinkled as garnish [1]. This delicacy is characterized by short shelf life therefore after harvesting it should be consumed quickly. For this truffle aroma is usually entrapped in oils, butter, and dairy products by using pieces of truffle as food additive. Cacciotta, Pecorino and Sottocenere are some popular Italian truffle speckled cheeses [1][16]. Different “truffle oils” are commercially available in specialty stores, but they are produced by using synthesized chemicals that they occur in truffle aroma fraction. In contrast some olive oil infusions are made with this fresh delicacy and they are usually consumed for dipping bread or drizzling pasta [16]. Truffle can be cooked in various ways as well [1]. For example, in the Arabian Peninsula truffle are eaten in different cooking manners: Fried, boiled with rice, or used as a replacement of meat in cooked vegetable dishes [17]. This fungus is used also for preparing sauces (truffle sauce, ketchup, mustard, sauce with mushrooms), soups, purees, spice (truffle salt), and it can be found preserved in brine and canned [16]. Besides the numerous gastronomic uses, truffle has been employed since antiquity for its presumed multiple biological actions. Desert truffle juice was used since the 10th century in the Arabian Traditional Medicine to cleanse the eyes and to eye inflammations while currently, in ethnopharmacology, it is known for aphrodisiac effects. Moreover, the boiled extract is used for the treatment of trachoma and as anti-inflammatory for eye diseases [17]. Modern scientific literature has evidenced that truffle can be considered a valuable therapeutic agent with antioxidant, anti-inflammatory, antimicrobial, antimutagenic, antitumor, aphrodisiac, and antidepressant activities [1][15][16][17]. This is the reason why it can be employed in pharmaceutical and cosmetic industry [16][18].

5. Tracing Truffles in the Soil and Interactions with Mammals

The high cost of truffles, arise from their difficult and labour intensive treasure-hunting dedication. In nature, truffles attract mammals ranging from wild pigs or boars to squirrels, which consume the fruiting bodies and contribute to spore dispersal [19][20][21][22]. Truffle hunters have traditionally used pigs and more recently trained dogs to localize the truffles underground. 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 volatile organic compounds (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. It was reported that a steroidal pheromone, 5α-androstenol, emanating from both black and white truffles with a characteristic musk odor, is responsible for attracting pigs [1][22][23][24]. However, it was later demonstrated that dogs and pigs were attracted not by 5α-androstenol but by dimethyl sulfide (DMS), another compound present in black truffles [19][22]. Because DMS is present in numerous truffle species, it might possibly, along with other unidentified compounds, act as an attractant for mammals in the wild. Therefore, DMS appears to be the key-odor compound for truffle location. Also, 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 [1]. Two other sulfurous and three C8 compounds are reported to be attractant for these truffle flies [1][25].

The unique flavor of truffles is one of the main reasons to get worldwide attraction as a food product. Previous studies have focused on screening and identifying the volatile organic compounds (VOCs) and characterized more than 200 VOCs in various truffle species. The major compounds responsible for the aroma in diverse truffle species are well

documented in our review which aimed to provide an update on research conducted on the analysis of active aroma components as well as the analytical techniques to identify them.

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