Food Waste Used for the Cultivation of Mushroom

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Pleurotus ostreatus (*P. ostreatus*) is considered a high-quality food, rich in proteins and bioactive compounds important for maintaining human health. Lately, a commonly used substrate for oyster mushroom cultivation—wheat straw, is more often replaced by alternative cellulose substrates originated from the agricultural and food industry. Utilization of wastes for mushroom cultivation has its added value: sustainable food waste management, production of high-quality food from low quality waste, as well as solving environmental, economic and global issues.

Keywords: Pleurotus ostreatus ; oyster mushroom ; food waste ; sustainability

1. Introduction

Different mushroom species are consumed in many countries around the world as traditional and functional foods [1], as well as a delicacy, for their specific flavor and texture properties [2]. On the other hand, public and scientific interest in mushroom secondary metabolites and bioactive components with their antioxidant, antimicrobial, antitumor, antiviral and immunomodulatory properties are increasing [1][3][4].

According to FAOSTAT data, total world production of mushrooms in 2020 was 43 million metric tons, with a total of 1.3 million metric tons in Europe. The leading producer was China with a total of 40 million metric tons. The most produced mushroom in the world is *Agaricus bisporus* (button mushroom), followed by *Lentinula edodes* (shiitake) and *Pleurotus ostreatus* (oyster mushroom) [2].

In general, mushroom species are categorized in three groups, according to Kalač [2]. This categorization is based on mushroom nutritional strategy. The first group includes mycorrhizal or symbiotic species, which form mutually favorable connections with the host trees. The second group, named saprotrophic species or saprophites, derive their nutrients from dead organic material. This species is the basis for commercial cultivated production. The third group, parasitic species, lives in non-symbiotic relationship on the other species.

The scientific classification of *Pleurotus* species belongs to the Kingdom of *Fungi*, Division of *Basidiomycota*, Class of *Agaricomycetes*, Order of *Agaricales*, Family of *Pleurotaceae* and Genus of *Pleurotus*, defined by the German mycologist Paul Kummer in 1871 [5].

One of the most consumed mushroom species, *P. ostreatus*, belongs to the “white-rot fungi” category, which produces the lignolytic enzymes laccase and peroxidase and makes them able to degrade lignin, in addition to cellulose. With regard to this feature, this species may be cultivated on a wide range of agro-industrial, food and cellulose wastes, as replacements for ordinary substrates used in industrial production [3][6], which affirms the fact that oyster mushrooms, among the diverse white-rot fungi species, represent high-quality food originating from low quality waste [7].

Apart from its delicate taste and texture, *Pleurotus* Genus represents a nutritionally rich food with a high content of crude proteins and dietary fiber [5][8]. Many studies have focused on the simplicity of the *P. ostreatus* cultivation, which is attractive for scientific and commercial utilization [8]. The study of Rodriguez Estrada and Pecchia [8] paid close attention to the cultivation method of *P. ostreatus*, including spawn preparation, substrate manipulation, as well as alternative cultivation methods, harvesting and the possibility of disease during fructification and mycelium running through the substrate. The focus on waste biodegradation and enzymatic activity of *Pleurotus* species was the highlight of the study of Sekan et al. [9], distinguishing the green potential of this fungi from this perspective. A new research study examined enzyme production and mycelium growth of *Pleurotus* spp. improved by green illumination [10], adding value to the sustainability aspect.

An aspect of food waste utilization was defined by Morone et al. [11] as a sustainable food waste management strategy with the main goal to isolate high value waste material. Essentially, isolating any food waste material from the household

or production process and using it as a raw material for a new production process that generates near zero-waste and makes an effective circular economy cycle is worthy of attention. Beneficial aspects of food waste recycling are diverse and include solving environmental [12][13], social [14] and economic [15] global issues.

In general, in addition to using waste as a resource for a new production process, a huge beneficial aspect of food waste recycling is the elimination of environmental pollution through the food chain [16].

2. *P. ostreatus*: Substrate Preparation

The substrate degradation capacity of *Pleurotus* spp. is related to its ability to secrete specific enzymes, such as laccases, cellulases, hemicellulases, peroxidases and xylanases in order to utilize needed nutrients, with no need for composting, which makes the commercial production relatively simple [17]. The substrate preparation procedure includes shredding substrate into small particle size (3 cm) and drenching it in distilled water for 24 h to reach 60% moisture content. Then, the substrate might be sterilized in polypropylene bags at 121 °C, 1.1–1.2 atm for 1–2 h [18][19][20][21][22][23] or 15 min [24][25], sterilized for 90 °C for 90 min [26], pasteurized at 60–80 °C into boiling water before the inoculation procedure [27][28], steamed over 100 °C for 4 h [29] or treated by aerobic fermentation: 65 °C for 36 h, fermentation for 12 h and aerial cooling for 24 h [30]. All other studies included substrate preparation in the same or similar manner.

After inoculation, different productivity parameters were measured during the mycelium running and harvesting period, such as mycelium running rate, time required for mycelium running completion, time required for primordia initiation and harvesting, total mushroom yield and biological efficiency [18], as well as organic matter loss, pileus, stipe diameter and stipe length [27].

3. Chemical Analyzes of Lignocellulosic Substrates Originated from Food Wastes

P. ostreatus, as a white-rot-fungi, decomposes cellulose, hemicellulose and lignin, as the main nutrient source for its mycelium growth [7]. Cellulose, hemicellulose and lignin are the major constituents of lignocellulosic wastes, which make them ideal for mushroom cultivation. The major source of cellulose is vascular plants' cell wall, and it is constructed from D-glucose units through β (1→4)-glucosidic bonds. Cellulose and hemicellulose belong to carbohydrates, and their bonds can be broken using acids or enzyme activity [31]. Additionally, the factors significant for mycelial growth, yield and efficiency of mycelium production include the range of C/N ratio, pH and moisture measurement [32]. According to Chang and Miles [33], most fungi require moisture content of the substrate between 50 and 75%, which supports maximum growth level. Carbon and nitrogen have an effect on mycelial exopolysaccharide production in submerged culture, which leads to the conclusion that fungal cell walls can be supported by nutritional signals or environmental stress [34]. According to Choi [35], nitrogen converts to ammonia during the fermentation process, which causes an interruption of mycelial growth at high amounts. Ideal values of nitrogen are between 0.5 and 2% [36]. Nitrogen-rich compounds, used as additives to mushroom cultivation substrate, result in higher mushroom yields and increased fungal metabolic activities triggered by the presence of extra nitrogen [37][38], which contradicts the claim that nitrogen is the cause of mycelial growth interruption [35]. On the other hand, the addition of nitrogen-rich materials may also lead to higher contamination risks by competitor microorganisms [39]. Substrate supplementation is the solution of successful fermentation of different lignocellulosic substrates intended for mushroom cultivation [40].

Regarding the physical properties of the substrate, granulometric profile and particle size specifies the surface area available for mycelial growth. Smaller particles result in substrate compression and lack of gas exchange and availability of substrate molecules for the hydrolitic enzymes responsible for mycelium growth, which limits mushroom yield [41].

Prior to inoculation, analysis of olive pruning residues mostly included pH measuring, electroconductivity, total organic matter content and moisture content and C/N ratio, besides cellulose, hemicellulose and lignin content. Atomic absorption spectrophotometry was used for mineral composition analysis (K, Ca, Mg, Na, Fe and Mn) [27]. Additionally, Sakellari et al. [42] analyzed the elemental composition of substrates with different ratios of olive leaves, olive mill waste and grape marc: Al, As, Ba, Cd, Co, Cr, Cs, Cu, Fe, K, Mn, Na, Ni, Pb, Rb, Sr, V and Zn. On the other hand, olive mill waste together with grape marc as mushroom substrates, were analyzed for total phenolic content, individual phenolic compounds, terpenics and squalene [23]. In addition to major constituent analysis, Melanouri et al. [43] included total nitrogen and organic matter determination of substrates containing grape pomace, coffee residue and olive pulp. Analysis of spent coffee grounds, corn and rice residues, respectively, involved the moisture content, total C and N content and pH measurement [44][45]. Adebayo et al. [29] included cellulose, hemicellulose and lignin analysis of palm and rice wastes, while Ma et al. [46] analyzed nutrient content of substrates consisting of used diaper and food waste, banana skin, coffee waste and

sugarcane bagasse, and C/N and total N content of coconut fiber, coffee husk and corn bran substrate mixtures [21]. Beer waste (spent brewery grains) was analyzed for pH and C and N determinations [25], while Rugolo et al. [47] and Fernandes Pereira et al. [48] included phosphorus, C/N ratio and humidity analysis. Sugarcane bagasse substrate analyses included cellulose, hemicellulose, lignin, total N, total C, P, Ca, Mg, Na and K [49], C/N ratio, S, H, pH and moisture content analysis [50], while rice substrate was additionally investigated for total K, P and heavy metals (Cr, As, Cd, Hg and Pb) [51]. Total N, organic matter, pH and electrical conductivity were investigated for soybean, olive and winery wastes [20][52].

4. Composition of Food Waste Substrates Used for Mushroom Cultivation

Olive waste substrates prepared in experiment by Koutrotsios et al. [19] contained olive pruning residues and two-phase olive mill waste (TPOMW) mixed with wheat straw in ratios 25, 50 and 75%, as well as the mixture of both wastes in ratios of 25 and 50%, while Fayssal et al. [27] used olive pruning residues only in a combination with wheat straw (1:3, 3:1). Extensive literature covers the preparation of substrate mixtures of olive and grape (wine industry) wastes. Thus, Koutrotsios et al. [23] and Sakellari et al. [42] used TPOMW, olive leaves, grape marc and wheat straw, as well as their combinations in ratios 3:1, 1:1 and 1:3. Olive and grape wastes, along with wheat straw, were used as substrates by Tagkouli et al. [53] and Tsiantas et al. [54] in the following combinations: wheat straw:grape marc (1:1), olive leaves:TPOMW (3:1) and wheat straw as control. Grape pomace as the only food waste substrate was utilized in the research of Doroški et al. [18], in the following mixtures with wheat straw: 100%, 80%:20% and 50%:50%.

Coffee waste covered in the literature was either prepared in a mixture with wheat bran and straw [43][55], while Carrasco-Cabrera et al. [44] utilized spent coffee grounds in a mixture with sawdust. Ling Ma et al. [46] included coffee and banana waste in a food waste mixture with the addition of diaper waste. Additionally, Nguyen et al. [56] used spent coffee grounds in the following formulations: 100%, in the ratios 50%:50% and 20%:80% with wheat straw and cardboard. Coffee and cocoa husk in the mixture with other wastes were used in the work of Lowor and Ofori [57]. Cocoa and palm wastes were utilized as substrates in the following studies: Mota da Silva et al. [58] mixed palm oil waste and cocoa almond peels in five substrates in different ratios; Fernandes Pereira et al. [48] used cocoa pod shells and beer waste residues, where beer spent grains varied between 10 and 90% (*w*/*w*), while cocoa waste was used as a supplement to the weight of 100 g. Palm oil wastes, bunches and shafts, were used in the other study [29] in combination with rice bran, sawdust and wheat bran, 100% of each substrate alone and in the combination of 50%:50% of bunch and shaft with sawdust, rice or wheat residues. Palm shell was used to produce biochar, then utilized as bio-fertilizer for oyster mushroom growth [59]. Biochar was mixed with rice bran and sawdust in three different mixtures, where biochar was weighted between 10 and 30 g, rice bran between 84 and 86 g and sawdust >850 g. On the other hand, different palm seeds in combination with shells of Brazil nuts and pine sawdust were studied as cultivation substrate by Vieira Bentolila de Aguiar et al. [60], while Zakil et al. [50] mixed empty fruit bunches, palm pressed fibers, fresh fruit bunches in combination with sugarcane bagasse and rubber tree sawdust, mostly in percentages 25%:75% and 50%:50%. Economou et al. [20] used a different approach and utilized spent mushroom substrate supplemented with wheat bran and soybean flour for a new mushroom cultivation process in order to obtain C/N ratios effective in cultivation. On the other hand, sunflower husks were used for oyster mushroom cultivation in a mixture with wheat straw (3:2) [30], while pure and degraded hazelnut husks (5%) were prepared in a mixture with the polymer matrix in the study of Duzkale Sozbir [26]. Peanut hulls and nuts mixed in different ratios (20%:80%, 50%:50%, 100%) were used in the study of Zied et al. [61] for *P. ostreatus* substrate supplementation. Beer wastes were used in the following studies: a pristine soil was mixed with previously immobilized spent brewery grains with fungal mycelium in a 1:5 ratio [25], malted barley (spent brewery grains) was used as supplementation for other agricultural waste substrates [47], while three categories of beer wastes: brewer's spent grain, hot trub and residual yeast was mixed with cocoa pod shells in different ratios [48]. Submerged liquid and solid-state fermentation were applied in the following studies: banana pseudostem and coconut fiber in the amount of 20 g were added into vials with Kirk's culture medium [22], and mycelial discs were inoculated for growth. This study covered the enzymatic activity determination. On the other hand, coconut oil cake, together with sesame oil cake, were prepared in conical flasks for mycelial inoculation in order to produce hydrophobin-like proteins [24]. Agro-cereal residues, including rice, corn and sugarcane wastes, were mostly prepared pure for fungi inoculation: rice straw and corn stubble were used in the study of Zarate-Salazar et al. [45], while Akter et al. [62] used rice husk and sugarcane bagasse additionally. Huang et al. [51] utilized rice straw, while Wiafe-Kwagyan et al. [63] additionally used rice bran and husk mixtures supplemented with different percentages of $CaCO_3$. Corn straw and sugarcane bagasse were mixed (50%:50%) in combination with plantain midrib in order to find out the best substrate for cultivation [64], while corncob alone and in the mixture with finger millet straw and bamboo waste was used in the following study [65].

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