

Review Article

Sunflower Seed Hull: Its Value as a Broad Mushroom Substrate

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Abstract

Sunflower (*Heliantus annuus*) seed hull (SSH), an abundant and cheap lignocellulosic residue from edible oil-seed industries, was evaluated as an alternative substrate for mushroom cultivation. Different edible and medicinal mushroom species were grown successfully, bringing a positive use of material that could be problematic for disposal. In this review we display different studies that linked the SSH with mushroom production and other derivations of its use as mushroom substrate.

ABBREVIATIONS

SSH: Sunflower Seed Hull; BE: Biological Efficiency; MP: Mushroom Production; P: Productivity; SS: Sunflower Seed

INTRODUCTION

Sunflower (*Heliantus annuus*) seed hull (SSH) is an abundant and cheap lignocellulosic residue of the local (Argentine) edible oil-seed industry. Twenty two thousand tones of SSH are produced as a residue of edible oil industries located at Bahía Blanca, Argentine [1].

SSH has been marketed for special purposes such as bedding material in poultry operations, fireplace logs and other high-fiber products, but these markets are limited. Also, it has been used sometimes as roughage for ruminants as cows and sheep's, but the high content of lignin makes it useless as animal food. More often the hulls are burned. Burying them in the soil has also been tried, but this practice is unsafe for the sanity of the fields because it could contain *Sclerotinia sclerotium*, a plant pathogen fungus [2]. Its disposal is an environmental problem because it degrades very slowly. During oil extraction procedure, raw sunflower seeds are transformed into oil and flour and seed hulls are produced as a by-product. SSH constitutes about 18-20% of raw seeds [3]. The main organic macro-nutrients of SSH are lipids, carbohydrates and proteins, with a 20-25% lignin of the total weight [4]. Reduced sugars are also an important part of the seed coating, amounting to about 25%. Total organic carbon coming from cellulose, hemicellulose and lignin account for more than 40%, making its C/N ratio quite high (54, own data). Lipids and proteins content are around 5% and 4%, respectively, and almost 3% of lipids are waxes, fatty acids and alcohols [5]. SSH chemical composition makes it an attractive material for growing lignocellulolytic microorganisms. White rot fungi,

basidiomycetes, are considered as the primary agents in nature for lignin degradation [6,7].

The biodegradation of lignocellulosic material by basidiomycetes is a cooperative process which involves the participation of oxygen reactive species (H_2O_2 , superoxide and hydroxyl radicals) and other phenoxide radicals together with ligninolytic enzymes collaborating with enzymes coming from the carbohydrate metabolism to degrade and assimilate lignocelluloses [8]. Mushrooms produce enzymes capable of degrading cellulose and hemicelluloses and oxidizing lignin. Thus, it resulted interesting to prove SSH as an alternative substrate for mushroom cultivation. Cultivation of different white rot edible and medicinal mushroom species has been growing in the last decades, mainly because of the easiness of its process, low inversion, and high productivity and increasing demand of the products due to their gourmet and medicinal values. Any lignocellulosic material could be a substrate for this purpose. This material is decontaminated by different methods, usually by hot steam (>60°C), and bagged to imitate a log. Mushroom's growth goes through two phases, spawn running -vegetative stage under darkness and a fixed temperature around 25°C- and fruiting -reproductive stage under light, oscillating temperature, ventilation and humidity- World mushroom production is headed by the white button mushroom (*Agaricus bisporus*), shiitake (*Lentinula edodes*), oyster mushroom (*Pleurotus* spp.) and portobello (*A. bisporus* brown). Also, there are other prominent mushroom species widely cultivated, such as reishi (*Ganoderma* spp.), *Schizophyllum commune*, maitake (*Grifola frondosa*), *Hericium erinaceus* and royal sun Agaricus (*Agaricus brasiliensis* syn. *A. subrufescens*, *A. blazei*).

Pleurotus ostreatus

Oyster mushrooms (*Pleurotus* spp.) possess an extracellular enzymes system and a strategy via free radicals, exhibiting a

great adaptability to degrade different kind of lingo cellulosic materials such as SSH. *Pleurotus* spp. grow on an extensive array of agricultural and forest waste products: straws as wheat, rye, oat, rice and barley, corn stalks, sugar cane bagasse, coffee pulp, banana leaves, cotton waste and cottonseed hulls, hardwood sawdust, paper by-products, soybean waste, palm oil by-products and agave waste [9]. In a previous work, SSH was evaluated for cultivation of oyster mushroom in boxes. The author obtained an average yield of fresh mushroom per kg dry SSH of 0.7-0.8 kg [10]. The first report of our group involving SSH as a main substrate for *Pleurotus ostreatus* cultivation proposed a novel substrate decontamination method. This method makes use of a metallic rotary drum with interrupted revolution under constant heating for relative short time (60 min), but sufficient to produce effective decontamination. It also allows a thorough mixing of the spawn into the mass substrate [11] (Figure 1).

It was demonstrated that this material alone, without supplementation, can be considered as a complete nutritive substrate to be colonized by *P. ostreatus* [12]. The most common supplement added to *P. ostreatus* substrate is cereal bran that stimulates mycelia growth and mushroom yield [13]. However, on SSH substrate supplemented with cereal bran, *P. ostreatus* mycelial growth was not increased [12]. With respect to the size of hulls, it was demonstrated that the particle size as it emerges from oil industry (average 12 mm) (Figure 2) is adequate for use as a media component [12]. Mycelial growth did not show significant differences when SSH were used in three particle sizes: 7, 10 and 12 mm (18 days for complete colonization). However, there were marked differences in fruiting and crop yield, being the largest particle size which produced the maximum Biological Efficiency (BE, (fresh mushroom yield/dry substrate weight) x 100), 76.5% in three flushes, with a first flush that represented 85% of the total (Figure 3), in comparison with smaller particle ones. So hull chopping is not necessary and would imply an extra cost [12].

Nutritional contents of substrate can be improved by nitrogen supplementation [14] and supplementing the substrate with Mn (II) shortens crop period and increases mushroom productivity [14]. In a report where a substrate containing SSH as main energy was used, five strains of *P. ostreatus* were evaluated in response to different levels of Mn (II) (0, 20, 100 or 200 ppm) and/or NH_4^+

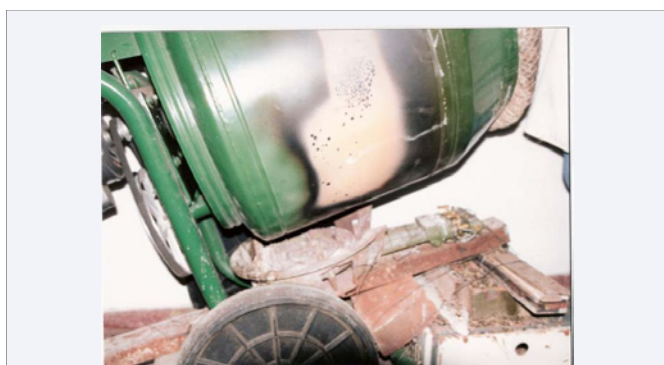


Figure 1 Concrete mixer adapted for the decontamination of sunflower seed hulls as substrate for mushroom cultivation.



Figure 2 Sunflower seed hulls as emerge from oil industry.



Figure 3 First flush of *P. ostreatus* on sunflower seed hull based substrate.

(0, 200, 500 or 750 ppm) [15]. Results showed that BE increased over control values and reached 60-112%, depending on the strain and the concentration of Mn (II) and NH_4^+ .

Lentinula edodes

Shiitake (*Lentinula edodes*) produces enzymes capable of degrading cellulose and hemi cellulose and oxidizing lignin [6,16]. The ability of shiitake to colonize substrates is limited by physico-chemical and nutritional factors [17]. When utilizing SSH as main substrate source for shiitake cultivation, it was obtained a daily production rate of 2 kg fresh shiitake/100 kg dry substrate for a 55 days production cycle and an accumulated BE of 108% [18] (Figure 4). Addition of wheat bran to the SSH-based synthetic log produced no significant differences on BE, mushroom production or productivity (P, BE/days from inoculation to last harvest). Mycelial growth rate measured by linear growth test [19] of SSH without supplementation was 2.4 mm/day, an interesting growth rate with regards to SSH supplemented with either wheat bran or poplar sawdust [2].

Ganoderma lucidum

Reishi (*Ganoderma lucidum*) is a medicinal mushroom that possesses biologically active substances like polysaccharides [20], steroids [21], and terpenoids [22] with different pharmacological activities as antitumor, hypopressor, antioxidant,

immunomodulating immunostimulating, hypocholesterolemic and hypoglycemic activities [23-30]. It is considered one of the most important medicinal mushrooms and it has been studied profoundly.

It was demonstrated that SSH can be used as the main energy and nutritional source in the formulation of a substrate for the cultivation of this mushroom [31] (Figure 5). SSH substrates containing 5% malt, showed a significantly higher productivity than those from other formulations, giving an average production rate of 0.5 fresh mushroom kg/100 kg dry substrate per day. In this case, production cycle was 5 days shorter than the other treatments.

In other work, the effects of supplementing SSH with olive oil and Cu (II) on mushroom production parameters and fruiting body total triterpenoids content were evaluated [32]. The addition of 1.5% olive oil increased total mushroom production (MP) by 21.7% (dry basis) in three flushes of mushrooms. Cu (60 ppm) increased the first flush daily productivity (MP day⁻¹) by both reducing the time needed to crop harvest and increasing the MP. When both supplements were combined, the MP at the first flush was 43% higher than control. An increment of 145-155% in the mushroom Cu content was obtained by addition of 60 ppm Cu to the substrate.

The utilization of sunflower seed (SS) is not restrained to



Figure 4 Shiitake mushroom first flush on a sunflower seed hull substrate.



Figure 5 *Ganoderma lucidum* carpophores grown on sunflower seed hulls.

mushroom solid fermentation. It could be observed that inclusion of sunflower seed broth (65 g.L⁻¹ seeds) in the formulation of a liquid culture for *G. lucidum* and *G. oregonense* could markedly improve mycelium and polysaccharide production [33]. Furthermore, one-gram *Ganoderma lucidum* dried mycelium grown in that liquid medium in the presence of 50 mg kg⁻¹ Cu or Zn showed a bioavailability of 19% Cu and 2% Zn of the recommended daily intake (RDI) for humans, using an *in vitro* simulated digestion system [34]. This study also reported that when reishi carpophores from SSH substrate were evaluated, the highest BE (23.2%) was reached when the substrate was enriched with 100 mg kg⁻¹ Cu and that time to crop fruiting was markedly reduced in the case of the substrate enriched with 50 mg kg⁻¹ Cu. However, Cu and Zn content obtained either before or after carpophores digestion from all metal enriched treatments, were substantially lower than those from metal enriched mycelia. The metal bioavailability was also low: 1.5% of the Cu RDI and almost negligible for Zn.

Grifola gargal, Grifola sordulenta and Grifola frondosa

Grifola gargal and *Grifola sordulenta*, two native edible mushrooms species, were evaluated in order to improve their cultivation both in solid agar and liquid media [35]. For both species, addition of milled SSH (0.4%) to the agar medium significantly increased mycelia growth diameter and biomass production. In the case of liquid culture of *Grifola sordulenta*, addition of 26 and 39% SS broth to the medium significantly increased mycelia biomass (almost three times) compared to the control, while *Grifola gargal* showed an increment of approximately 100%.

Artificial cultivation of these mushrooms was achieved using SSH as main substrate component [36] (Figure 6). The residual substrate produced after solid state fermentation was enriched in proteins with good laccase activity and with lower lignin content than original substrate. SSH pre-treated with dilute H₂SO₄ or supplemented with Mn (II) or Zn (II) salts were found to improve mycelia colonization process.

In the case of *Grifola frondosa* (known as Maitake), it was demonstrated that its mycelium could accumulate Cu and Zn when grown in a liquid medium containing 26% SS broth [37]. Solubility of Cu and Zn from metal enriched mycelia was 32-33% and 0.7-3.5% of the RDI for Cu and Zn, respectively, in one gram of mycelium. In addition to the utilization of these mycelia as a nutritional supplement, the authors suggested the possibility of an additional cosmeceutical application.

Hericium erinaceus

Lion's mane mushroom (*H. erinaceus*), a highly appreciated edible and medicinal mushroom, was evaluated to be grown in a SSH substrate (Figure 7). The mycelial growth rate in substrates possessing different SSH sizes showed a higher mycelial growth rate with the larger SSH size (as disposed of by the regional oil-seed factory without additional process) [38]. Adding growth-limiting mineral nutrients such as Mn (II) (20 or 100 ppm) and/or NH₄⁺ (200 or 500 ppm), increased the mycelial growth rate by 8-16%. However, SSH without supplementation achieved good



Figure 6 Grifola sordulenta carpophores grown on sunflower seed hulls substrate.



Figure 7 Hericium erinaceus grown on sunflower seed hull substrate.

BE (37.4%) and P (0.7% day⁻¹) values, constituting a very good basal substrate, so this substrate by itself can be considered a very good source of energy and nutrition for *H. erinaceus* growth and development.

Schizophyllum commune

This is a cosmopolitan mushroom which has been considered in the Orient as a healthy food and an effective medicine to treat several diseases [39]. It was reported at *S. commune* production assay on SSH synthetic logs, in absence or presence of wheat bran in a three harvest cycle [40] (Figure 8). Accumulated BE and P (48.3% and 1.6% day⁻¹, respectively) on SSH based substrate containing 7.5% wheat bran were significantly greater than those obtained on SSH alone (40.7% and 1.1% day⁻¹, respectively).

Agaricus bisporus

Cultivation of *Agaricus bisporus* (white button mushroom or champignon) using a small-scale composting system for substrates mixtures containing SSH was described [41,42] (Figure 9). SSH demonstrated to be appropriate for a good quality compost formulation (adequate color, odor, N content, pH, humidity content and actinomycetes presence). It was the first study of *Agaricus* production on a substrate based on SSH (51.4% SSH, 40.0% wheat straw, 3.8% wheat bran, 1.2% urea, 1.2% ammonium sulphate and 2.4% calcium sulphate).

Agaricus brasiliensis

Agaricus brasiliensis (syn. *A. blazei* Murrill, *A. subrufescens*) is one of the most promissory and studied mushrooms species, due to both its nutritional and medicinal value, besides its peculiar characteristics of flavor, almond fragrance and excellent texture [43]. Its cultivation on SSH was evaluated on both composted and non-composted substrates.

SSH IN COMPOST SUBSTRATE FORMULATION

Substrate formulation containing SSH to obtain compost capable to support a complete growth cycle of *Agaricus brasiliensis* was evaluated [44]. Formula consisted in 50% SSH, 41% wheat straw, 4.5% wheat bran and supplements and additives. Different types of containers: polyethylene bags and plastic trays were also evaluated. This compost resulted appropriate for *A. brasiliensis* cultivation, yielding BE ranging from 30 to 47%, depending on the container and substrate mass, being highest with polyethylene bags containing 2.5 kg substrate. This mushroom degraded SSH lignin during the cultivation, having the access to nutrient sources of cellulose and hemi cellulose.

SSH in non-composted substrate

Cultivation of *A. brasiliensis* on axenic substrates was studied. Substrate formula consisted in SSH, *P. pulmonarius* spent substrate, or their combination, in the absence or presence of different supplements (vermicompost, peat or brewery residues). Yields results were similar to composted substrates [45] (Figure 10). The lignin-hemi cellulose fraction was preferentially used as it was shown by substrate changes composition measured at the end of two flushes.



Figure 8 Schizophyllum commune on SSH based substrate and wheat bran (7.5%).

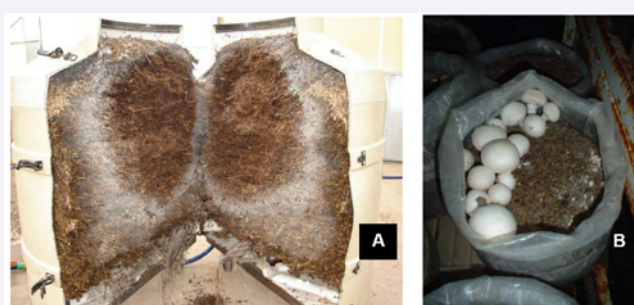


Figure 9 (A) - Small-scale composting system for substrates mixtures containing SSH for (B) - Agaricus bisporus production.



Figure 10 *Agaricus brasiliensis* on non-composted substrates containing SSH with an equal part of *P. pulmonarius* spent substrate.

Agaricus brasiliensis laccase production obtained either in composted or non-composted SSH and wheat straw was studied in order to evaluate the remaining substrates as a potential source for the extraction of high valued ligninolytic enzymes like laccases [46]. Cu solutions incorporation (100 or 200 ppm) on top of the casing layer (composted substrate) or as part of the formula (non-composted substrate) stimulated laccase production. Only the addition of 100 ppm Zn had a positive effect on lac cases and mainly in composted substrates.

Liquid cultivation of *A. brasiliensis* mycelium in nutrient medium containing SSH and 100 or 200 ppm of Cu or Zn, during only 4 days, could be adequate for the production of a nutritional supplement [47]. This *A. brasiliensis* Cu or Zn enriched mycelium, besides the medicinal and nutritional attributes characteristic of the species, would provide a concentrated source of essential minerals in a potentially bio available form, allowing coverage of 60-98% and 9-11% of the RDI for Cu and Zn, respectively, with only 1 g of mycelium.

Hypsizygus marmoreus

A formula that included SSH (SSH 38%, soft almond shells 38% and wheat bran 24%) was studied among others for the cultivation of *H. marmoreus* [48]. However, the BE obtained in a month from 0.8 kg polypropylene bags was very low (6.1%) compared to the other substrates studied.

Other medicinal mushrooms

For small-scale cultivation, SSH was used as alternative substrate for medicinal mushroom cultivation. SSH was one of the most suitable substrate for the cultivation of *Agrocybe aegerita*, *Auricularia polytricha*, *Flammulina velutipes*, *Ganoderma lucidum*, *Hericium erinaceus*, *Lentinula edodes*, and *Hypsizygus marmoreus* selected strains as well as for the gourmet Oyster mushrooms, including *P. ostreatus*, *P. citrinopileatus*, *P. sajor caju* and *P. djamor* [49]. Mushroom yield was higher in comparison with supplemented sawdust (beech) substrates in various SSH combinations.

Derivations of SSH utilization as mushroom substrate

Fungal enzymes extracted from *Pleurotus pulmonarius* (*sajorcaju*) mycelia cultivated on liquid media containing 65 g/L SS were utilized to eliminate aniline/phenol from wastewaters

[50]. They were able to polymerize and precipitate them from aqueous solutions, obtaining soluble polyaniline or insoluble polyphenol.

The potential use of *A. brasiliensis* SSH based spent compost as a remediation tool for cleaning metsulfuron methyl polluted soils was evaluated [51]. The complex enzyme fractions extracted from the spent mushroom compost had the ability to degrade metsulfuron methyl during the right incubation times to compounds with no or lower phytotoxicity than this herbicide.

Biodegraded SSH resulted from mushroom cultivation was studied. Analytical composition of a SSH based compost discarded after two flushes of *A. brasiliensis* showed that the average content of cellulose and hemi cellulose were reduced by 32% and 54%, respectively. Lignin content decreased by 47%, compared to initial values at the start of the composting process [44]. Biosurfactant production conditions by *P. djamor* on renewable substrates were optimized. Three different agro industrial wastes were used as solid substrates. According to surface tension values the best solid substrate was SSH as it contains hydrocarbon that activates bio surfactant production due to its insoluble property [52]. Residual substrate from *G.lucidum* cultivation was used to make biodegradable containers (Ganocetas) for growing horticultural seedlings [53]. These Ganocetas did not affect either the germination of 15 vegetables species or tomato production whereas it improved the growth and vigor of six species including two varieties of tomato.

DISCUSSION AND CONCLUSION

Although SSH is not used in human nutrition because of the presence of high content of recalcitrant lignin, it can be employed as main substrate for edible and medicinal mushrooms cultivation. Mushroom species diversity that can be cultivated in SSH substrate is large. We have compiled different kinds of mushrooms, primary or secondary decomposers, edible or medicinal: *Agaricus bisporus*, *Agaricus brasiliensis*, *Lentinula edodes*, *Pleurotus* spp., *Schizophyllum commune*, *Grifola* spp., *Hericium erinaceus*, *Ganoderma* spp., *Hypsizygus marmoreus* and many more. Furthermore, addition of Cu and Zn to SSH substrate to obtain better quality mushrooms was discussed. Many other mushrooms could be cultivated on SSH substrate with or without supplementation.

In locations where SSH is an abundant residue of edible oil-seed industry which needs to be disposed, its utilization as substrate for mushroom cultivation appears to be an interesting alternative to obtain good quality food and an economic benefit from an agro industrial waste that has no value.

We also showed alternative uses of residual SSH substrate after mushroom production for bioremediation or horticultural purposes.

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