Application of Brewer Spent Grain from the Beer Industry as a Unique Substrate for the Cultivation of Pleurotus Ostreatus Mushrooms

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Abstract

In Peru, the beer industry produced 998 million liters during 2020, and its manufacturing process generates various agro-industrial waste such as Brewer Spent Grain (BSG), equivalent to 80% of all agro-industrial waste generated approximately 570 million tons in the year 2020. This research aims to establish whether BSG as a residual product can function as a substrate for the cultivation and production of *Pleurotus ostreatus mushrooms*, offering an alternative to existing commercial substrates by evaluating the performance of its characteristics such as pH level, presence of ash, nitrogen, its biological efficiency (kg of mushroom/kg substrate), its productivity (biological efficiency/cultivation time), among other factors. An experimental design of mushroom cultivation was carried out according to the conventional assisted fruiting method in polyethylene bags. The laboratory analyses concluded that BSG as a substrate for the cultivation of *Pleurotus ostreatus* had a biological efficiency of 27,25% lower than the results of other substrates such as corn and coffee pulp, which have a biological efficiency of 68% and 70% respectively; although it presented a productivity of 0,605 compared to similar studies that on average had a productivity of 0,75. The study showed that BSG is a good substrate for the cultivation of *Pleurotus ostreatus* and it is expected that its performance can improve when used as a mixed miscellaneous substrate, offering a new circular economy alternative for the brewing industry.

Keywords
Circular economy, Oyster Mushrooms, BSG, Pleurotus ostreatus, Agro-industrial Waste, Brewing Industry, barley.

1. Introduction
The beer industry in Peru has shown constant growth, in 2020 a production of 998 million liters was estimated (Castillo 2021), and 47% of per capita alcohol consumption in Peru corresponds to Beer (Quispe 2018). Beer production generates a high volume of solid waste, derived from different stages in the brewing process. Of the most relevant wastes are the following: Brewer's Spent Grain (BSG), is the barley left over from the malting process, which represents 80% of industrial waste; Hot tub is the name given to the remaining hops from the production process and Brewer's Spent Yeast (BSY) is the remaining yeast from the fermentation process (Dos Santos et al. 2015).

It is important to mention that on average to produce 22 liters of craft beer Ale, 12,5 kg of barley is required, which will end up becoming BSG; this amount varies depending on the recipe used by the producer (Mencia and Perez 2016). One of the main differences between industrial and artisanal brewing is the amount of barley used per production batch, with the artisanal method requiring more than double the amount of barley used per batch produced;

representing a great problem for the management of this agro-industrial waste, since in Peru there are around 100 registered craft breweries (Pellegrin and Plasencia 2021).

The reasons why BSG is a good substrate for mushroom cultivation are its great capacity to absorb and maintain moisture, its considerable lignin content (Khidzir et al. 2010), and its high protein and natural fiber content. (Rathore et al. 2017), on the other hand, although no less important its density and porosity, are factors that influence and improve fungal growth (Mussatto 2014). Despite this, BSG presents some problems when used as a substrate, mainly problems with long-distance transport, due to its premature decomposition, which takes around 7 to 10 days before becoming unusable waste (El-Shafey et al. 2004), and the lack of processes developed to take advantage of it for this purpose, mostly opting for more immediate and shorter processes (Gregg et al. 2020).

Regarding *Pleurotus Ostreatus mushrooms*, they are the second most cultivated species of edible mushrooms worldwide, with 19% of the total mushroom production (Royse et al. 2017), in the Latin American region, Mexico represents the country with greater volume, with 80,8% of the total produced (Romero et al. 2018). *Pleurotus ostreatus*, also known as oyster mushroom, has a high protein content, comparable to eggs or milk (Demissew 2019), is one of the few sources of vitamin D2 in foods of plant origin (Mattila et al. 2002) and has a high natural fiber content, ranging from 12,39% to 29,75% (Ritota and Manzi 2019). At the same time, the advantages in production lie in its short colonization period (between 40 and 50 days) and its resistance to attack by insect pests (Hoa et al. 2015). On the other hand, these mushrooms also have many applications in the pharmaceutical industry, mainly focused on counteracting kidney problems (Rathore et al. 2017). In addition, they are well recognized for the quality of degrading lignocellulose, due to which various substrates generated from agri-food waste manage to improve their growth and development of their fruiting bodies, since they are essentially composed of lignocellulosic material (Ritota and Manzi 2019), Thanks to this quality it is possible to use the BSG and propose a new use for this waste, giving it the value of raw material.

1.1 Objectives

The general objective of the research is to develop the application of Brewer Spent Grain from the beer industry as a unique substrate for the cultivation of *Pleurotus ostreatus mushrooms.*
The specific objectives of the study are:

- Analyze the literature review to establish various alternatives for various substrates for the cultivation of *Pleurotus ostreatus mushrooms*.
- Evaluate the application of Brewer Spent Grain from the beer industry as a substrate for the cultivation of *Pleurotus ostreatus mushrooms.*
- Compare the efficiency of BSG as a substrate with other agro-industrial waste most commonly used in mushroom cultivation.

2. Literature Review

The analysis of studies that use various types of substrates was carried out, such as the following:
The first selected study uses coffee pulp as a substrate for the cultivation of oyster mushrooms. In this study, the essential factors for the production of oyster mushrooms are evaluated in depth using coffee pulp as a substrate. Among these factors, is the importance of the pH level in the substrate used, which should be between 5 and 6, this does not mean that a higher pH is not effective, neutral levels close to 7 are also very effective in substrates based on wheat and oats (Dedousi et al. 2023), as well as its level of carbon and nitrogen, which represent the main food source for the fungus. The experimentation was carried out on 3 different substrate compounds, the first compound used only white pellet shavings, the second composition of the substrate is based on a mixture of shavings and coffee pulp and the last one used only coffee pulp, of the three the substrate The most efficient was the combination of coffee pulp with white pellet shavings, obtaining more than 90% biological efficiency, this composition being the most efficient of the three substrates (Mendoza 2020).

In the following study analyzed, mushroom cultivation was carried out on waste from the wood industry, using three different types of compounds, the first substrate was sawdust type with small chips, the second substrate is chip type of around 0,1 m and the last It is of the sawdust sheet type, of these three the most efficient was the chip type, which allowed correct proliferation and fruiting of the fungus, on the other hand, the substrate composed of sawdust sheets did not allow the proliferation of the fungus on its surface. due to its lack of porosity (Martinez et al. 2008). Less dense

substrates such as rice husk reached a biological efficiency greater than 90%, with fruiting bodies of 13.8 cm in length, quite acceptable by commercial standards (Mobou et al. 2023).

Another research mentions the importance of the percentage of nitrogen in the substrate with values around 1% which allowed biological efficiencies of 133% to be achieved. These results were obtained using beech wood chip substrates, rice bark (Dedousi et al. 2023b).

The high protein content of oyster mushrooms is a fundamental value to consider. Studies show that the composition of the substrate has direct effects on its protein percentage. Substrates based on wheat bran reached crude protein levels of 19,14% and 5 IU/100g of Vitamin D, these being nutritional levels for products not derived from animals (Elkanah et al. 2022).

The method selected for the experimental part of mushroom production is the conventional assisted fruiting procedure in polyethylene bags, for which the substrate is required to be at approximately 25 °C and have a humidity greater than 80%. Once the substrate is inoculated, the bags are placed in a dark environment at 18 °C for a period of 20 to 30 days. After this period, the bags are moved to a more illuminated area to induce a change of conditions that improve fruiting. of the fungus that lasts 4 to 5 days The application of the use of BSG as a mushroom substrate is one of the proposals for the problem raised, however, there are other ways to reuse this agroindustrial waste to generate value from it. BSG has different nutritional components that make it possible to use it for the production of beverages and foods. A study showed BSG can be used as an ingredient in the preparation of beverages and breads with acceptable chemical and nutritional properties, resulting in high protein levels of the beverages produced; on the other hand, in the case of bread with replacement of wheat flour by bagasse residue by approximately 10%, the fiber content could be increased by double. Other studies achieved similar results in the fiber content of bread by enriching 3% of food additives such as nejayote with respect to white corn (Acosta-Estrada et al. 2014). On the other hand, in order to validate whether the constant consumption of BSG significantly influences the body, research carried out an experiment where a group of people consumed 8,3 g of BSG daily for 8 weeks in a row, where the results were obtained that the consumption of BSG is well tolerated by our digestive system and improves dietary fiber intake, but does not significantly affect CVD risk factors (Schmidt et al. 2023).

3. Methods

3.1 Design of the Investigation

The design of this research is experimental, where different tests were carried out on a batch of *Pleurotus ostreatus mushroom* produced, in order to determine the efficiency of the Brewer Spent Grain (BSG) by analyzing indicators such as: pH level, percentage of ashes, the percentage N, which will be measured as an experimental subject. The BSG used for this study comes from the production of an Ipa-type beer for which 6 different types of malt were used, the malts being found in greater proportions: Pale Ale, Munich and in lesser proportions: Caramel Amber, Caramel Plis and Caramel Munich II.

For the experimentation phase, which consists of four stages, 5 bags with a capacity of 4 kg each were grown in which 3,7 kg of BSG and 300 g of mycelium were placed. The first stage is called inoculation, in which the bags are filled with BSG and mycelium alternately, the second stage is called colonization, where the bags are left for 27 days in an environment of complete darkness to provide the necessary conditions. to promote the correct colonization of the substrate, later in the Fruiting stage, the bags are moved to a place with a slight presence of sunlight (approximately 200 lux), during this stage inter-daily irrigation will be applied to the bags, finally carries out the harvest, which consists of extracting the fruiting bodies from each of the bags. The main indicators of mushroom production are calculated at the end of the entire cultivation process, such as:

$$\boldsymbol{Biological\ Efficiency} = \frac{\boldsymbol{kg\ mushroom\ (fresh\ weight)}}{\boldsymbol{kg\ of\ substrate\ (dry\ weight)}}$$

$$\boldsymbol{Productivity\ Rate} = \frac{\boldsymbol{Biological\ Efficiency}}{\boldsymbol{Cultivation\ Time}}$$

To carry out the substrate characterization analysis, 400 g of BSG (100 g per experiment) were sent to the laboratory located in the district of Lince, in the department of Lima, in which studies of ash, humidity, nitrogen and pH were

carried out. The methods used to measure the variables comply with the Peruvian technical standards mentioned in table 1.

3.1 Methods and materials used to search for information

3.1.1 Materials

For the cultivation process, 60 x 30 cm polyethylene bags are used as study units. In turn, two scales were required, one in order to weigh the mycelium in quantities less than one kg and the other with larger measurements used. In weighing the substrate, finally, lime (CaO) a fan was used in order to maintain adequate conditions in the incubation room.

Table 1. Peruvian technical standards for analysis

Analysis	Method
Ash	NTP 205.004 (2017)
Humidity	NTP 205.002:1979 (Revised 2016)
Nitrogen	NTP 205.005:1979 (Revised 2011) / AD 1:2012
Ph	AOAC 943.02 (2019). pH in flour

3.1.2 Equipment's

In order to carry out the study of the variables to be analyzed, it was necessary to use different laboratory equipment, among them are:

- Potentiometer: Instrument that determines the difference in electrical potential. Currently, it is used to determine the pH level, in the case of this research it was used to measure the pH of the BSG, since this variable directly influences the metabolism of the mycelium (Ruiz 2011).
- Kjeldahl distiller: To determine the organic nitrogen existing within the substrate, the Kjeldahl distiller was used applying the Kjeldahl method, which consists of 3 processes: Digestion, Distillation and Titration. In the first, the organic nitrogen of one of the substrate samples was converted into NH4+, in the second step the NH3 obtained was distilled and then collected and finally in the titration the existing nitrogen was determined (Mera 2015).
- Terrigen Muffle: Terrigen muffles are furnaces that can generate a temperature of 1,200 °C. This equipment is widely used in laboratories for different tests, among them is the ash percentage, in the case of our research it is determined the percentage of ash from the BSG.

4. Data Collection

The data collection process was developed at the end of the experimentation phase where the BSG used in the project was obtained, as well as the *Pleurotus ostreatus mushrooms* produced, the data to be obtained are the production time, the final weights of the BSG used and fruiting bodies generated, ash, moisture, nitrogen and pH levels. One of the precautions that must be taken in the experimentation site when extracting the final products is the use of a mask, gloves and safety glasses, in order to maintain the safety of the process and the spores generated by fungi, which can affect health with mild infections or generate an allergic reaction. Finally, to obtain information on specific indicators, the results obtained from the specialized laboratory were obtained. With the data obtained, an analysis of the following variables was carried out to determine if BSG is a good substrate for the cultivation of fungi.

- **pH (Pondus Hydrogenii)**, The pH indicates the degree of acidity of a compound or substance, having a range from 0 to 14. There is an ideal pH range for the growth of Pleurotus Ostreatus in substrates, this value being from 5 to 6, but due to the nature of the Oyster mushroom it is capable of growing in a range of 4 to 7. If the pH of the substrate reaches a value lower than 4, the fungus will be unable to develop (Zárate 2015).
- **Ashes**, Ashes are defined by some studies as inorganic waste that is the result of burning or incinerating organic matter, commonly using a muffle at temperatures between 500 and 600 °C for one day. At the end, it can be quickly identified when there are no longer carbonaceous particles (black) and the resulting ash product is only white or uniform gray. Finally, it is collected and weighed to calculate the percentage of ash

present. This percentage represents the total mineral content in foods and is expected to be a high percentage at the substrate level (Márquez 2014).

- **Percentage of N**, fungi of the Pleurotus family have the ability to fruit in substrates with low levels of nitrogen, due to this it has been suggested by different studies that this genus of fungus has the ability to fix atmospheric nitrogen. Despite this, nitrogen is considered a primary macronutrient that favors the growth of the fruiting bodies of fungi (Muñoz 2017).
- **Biological Efficiency**, In each of the batches obtained from Pleurotus Ostreatus mushrooms, this variable will be calculated, which is determined from the percentage of Oyster mushrooms produced based on the dry matter of the substrate used (Zárate 2015).
- **Productivity of Oyster Mushrooms**, the colonization time of the BSG is a fundamental variable to determine the productivity of the crop obtained based on time. This will allow us to have an average productivity of the different batches of Oyster mushrooms to be obtained, from which the development of the large-scale project can be further developed.
- **Spent substrate**, the problem raised is the high generation of BSG by the brewing industry. The best way to measure how much can be reduced is by calculating the substrate that is used or consumed during the mushroom production process. This indicator determines the amount of BSG decomposed at the time of generating fruiting bodies.

5. Results and Discussion

5.1 Numerical Results

The experiments used to obtain the indicators linked to the characteristics of BSG as a substrate were carried out according to the methodology explained previously in the laboratories.

- The pH of the BSG was determined, the experiment gave a result of 4,21. The tests were carried out at a constant temperature of 20 °C and demonstrate a more acidic pH than expected.
- The amount of ash presents in the BSG, the sample examined, resulted in 2,86 g per 100 g of BSG. It should be noted that the results are carried out on dry mass.
- Finally, the proportion of N present in the substrate was determined; the tests resulted in 2,71 g per 100 g of BSG, results based on the dry mass of the sample.

To determine the efficiency of the mushroom production process, the traditional method of assisted fungal inoculation in polyethylene bags was followed as explained above, taking into account that 5 bags were studied for a total period of 45 days, 27 days of colonization and 18 days of fruiting, in which the following results were obtained:

The calculation of biological efficiency was obtained from the amount of BSG used as a substrate for each of the 5 bags of the batch studied, taking into account that the humidity present in the BSG is 70,5%, obtaining on average of all bags 27,25%, it should be noted that, during the fruiting period, which lasted 18 days, two harvests were carried out, obtaining the following results presented in Table 2.

Table 2. Biological efficiency of BSG

Bags (Unit)	**Bag A**	**Bag B**	**Bag C**	**Bag D**	**Bag E**
Dry sustrate (kg)	1,09 kg	1,09 kg	1,09 kg	1,09 kg	1,09 kg
Mushroom produced (g)	318 g	277 g	325 g	260 g	307 g
Biological Efficiency (EF)	29,13 %	25,38 %	29,78 %	23,82%	28,13 %

The second indicator of this variable is the productivity of Oyster Mushrooms. To calculate this indicator, a total period of 45 days and a biological efficiency of 27,25% were taken into account, resulting in a general productivity of the experiment of 0,605. On the other hand, the individual productivity of each unit of analysis is presented in Table 3

Table 3. Productivity of each study unit

Bags (Unit)	**Bag A**	**Bag B**	**Bag C**	**Bag D**	**Bag E**
Biological Efficiency (EF)	29,13 %	25,38 %	29,78 %	23,82%	28,13 %
Productivity	0,647	0,564	0,662	0,529	0,625

The last indicator is the amount of substrate spent; After the final harvest, the bags were weighed, resulting in 18,5 kg of spent substrate, a mixture of substrate and fungus, representing 92,5% of the initial weight.

5.2 Proposed Improvements

Las pruebas realizadas han brindado la posibilidad de generar futuros estudios, considerando los siguientes criterios:

- Constantly control temperature and humidity variables, as well as ensure the safety of the growing environment, this allows better development of fruiting bodies and can prevent the appearance of pests that damage crops.
- Compensate the pH level of the BSG with some other substance, which would allow better colonization of the mycelium and increase the productivity of the process.
- Use a substrate of higher density mixed components, which compensate for the dimensions of the BSG and its PH level.
- Perform laboratory tests during the first week of obtaining the BSG in order to obtain the most accurate data on its initial composition.
- Carry out the inoculation of the substrate as soon as possible and at a temperature below 40 °C, greatly reducing the possibility of contamination by external agents and avoiding using the pasteurization process.

5.3 Validation

The first indicator studied was the pH level of the BSG, obtaining a level of 4.21, which is a value that is too acidic compared to other studies, where substrates with pH levels between 6,7 and 7 were used (Hoa et al. 2015), despite this, *Pleurotus Ostreatus* has the ability to grow in substrates with a pH between 4 and 7, although these extremes significantly reduce the efficiency of the crop.

As for the second indicator referring to the characterization of the substrate, it is the amount of ash present, being 2,86 g per 100 g of dry mass (DM), this value is relatively low compared to other studies that used quinoa residues as substrate, which presented 9,9 g per 100 g of DM (Maccapa 2021).
The last indicator referring to the substrate is the percentage of Nitrogen present in the dry mass, the result being 2,71%, which is a high value compared to other substrates based on sawdust, which present values between 0,8 and 1,2% (Hoa et al. 2015).

With respect to production, the first and perhaps the most important indicator is the biological efficiency obtained, which was 27,25%, this value is low considering biological efficiencies ranging from 68% to 72%, coming from crops that They use corn and coffee pulp (Prieto 2017).
The next indicator is the productivity of the crops, this being 0,605, a quite acceptable value compared to other works that obtained 0,75 (Mendoza 2020), despite this, this value can be greatly improved in more controlled cultivation environments.

Finally, with respect to the spent substrate, it should not be discarded, since the most inoculated parts, which present a higher concentration of mycelial branches in the substrate, can be used to inoculate new cultures, and most of the leftover substrate It can be used as animal food thanks to its high protein and fibrous content.
Based on the results, if this project is implemented, a new purpose would be provided to the BSG, leaving aside its treatment as waste and implementing the circular economy system, it would take the role of raw material to generate a new product that provides benefits. both economic and social, in turn, the amount of waste discarded would be reduced, reducing the environmental impact of the beer production process.

6. Conclusion

From the research, we can conclude that it was indeed possible to determine the efficiency of BSG as a unique substrate for the cultivation of oyster mushrooms, however, this efficiency was not as expected, and has certain deficiencies

compared to other agro-industrial waste. Despite this, BSG still has good characteristics to be used for this purpose and as the main component for some mixed substrate.

From the experimental study carried out, it can be concluded that BSG can be used as a substrate for the cultivation of mushrooms, thus allowing small, medium and large brewing companies to take advantage of this agro-industrial waste by applying circular economy concepts and reducing the pollution levels generated. by their companies.
It can be concluded at the end of the research that by introducing a new substrate option such as BSG to the market for mushroom cultivation, it would promote the industry and trade of edible mushrooms, facilitating access for consumers to purchase this healthy product. in addition to reducing the cost of production, since an agroindustrial waste that is generated in abundance is used as raw material.

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