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Research Article

Cultivation and Characterization of Oyster Mushroom and Its Application as Confectionaries

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ABSTRACT

This study represents the cultivation of oyster mushrooms at room temperature, preparation of mushroom powder and ready-to-eat cookies from the combination of cocoa powder and mushroom powder with long shelf life. Nowadays, people are facing difficulties in meeting their daily nutritional needs through ready-to-eat foods, prompting the exploration of white oyster mushrooms as an ingredient for making cookies, potentially serving as an emergency food product. Mushroom farming is gaining popularity day by day.The mushrooms have lots of potential to be used in the diet for taking advantage of the nutraceutical properties of bioactive compounds due to lower fat and higher protein substance. The drift is absent from the canned item toward new and dried mushroom sales. Oyster mushrooms are popular due to their nutritional, medicinal, and potential commercial value. They can be cultivated using agro-waste raw materials such as rice straw and wheat straw. We prepare mushroom cookies that provide protein, carbohydrates, fat, and crude fiber. A wide selection of shapes and sizes, high digestibility, high energy value, relatively low production costs, convenience, and long shelf life contribute to their popularity.

Keywords: Oyster mushroom, Cookies, Processing, Nutritional value.

INTRODUCTION

The main aim of study is to cultivate an oyster mushroom at room temperature and prepare mushroom powder and ready-to-eat cookies from the combination of cocoa powder and mushroom powder with a long shelf life. The oyster mushrooms were included in the genus Pleurotus by German mycologist Paul Kummer in 1871 (Stamets 1993).[1] The oyster mushroom was first cultivated by Flank in Germany in 1917.[2] The Romans thought mushrooms were the food of the gods. Mushroom cultivation is an indoor activity process that uses waste material to produce nutritious food. Around 2,000 species exist in nature but around 25 are widely accepted as food.[3] The global mushroom industry has expanded very rapidly in the last two decades through the addition of new types of mushrooms for commercial cultivation.[4] India has a wide range of agro-climatic conditions and is largely an agricultural country with a cultivated area of about 4.37%, generating about 620 million tons of agro-waste annually. Mushroom cultivation not only helps recycle agro waste but also fills the nutritional gap prevalent among a large population of India.[5] Oyster mushrooms can be cultivated naturally. It is not harmful to nature, because it can be cultivated by using inorganic waste or farm waste like rice straw, wheat straw, paddy straw, etc.[6] The basic requirements for mushroom cultivation are manure/compost, spawns, right temperature, and humidity. Favorable growing conditions involve 80 to 90% relative humidity, ample ventilation, a temperature range of 20 to 280°C during spawn run and 12 to 180°C for reproductive growth. Initially, for a week, temperature must be maintained at 23 ± 2°C and then it can be reduced to 16 ± 2°C for subsequent weeks. The CO_2 concentration should be 0.08 to 0.15 %. If the above-stated conditions are maintained appropriately, the pin heads start to appear within a few days and progressively mature into the button stage.[7] The steps involved in cultivating oyster mushrooms are substrate preparation, spawning of substrate, incubation, fruiting, and harvesting.[8] Edible mushrooms are fungi that can be seen with the naked eye and are relatively easy together by hand. Edible mushroom contain various vital nutrients and are the best food item with high nutritional value. The types of mushrooms differ in colors, shapes, surfaces, and activities.[9] To choice good quality oyster mushrooms, look for clusters that are firm, fresh, and smooth. Avoid mushrooms with holes running down the stem, which means they are likely to bug-eaten. The external appearance of oyster mushrooms have been characterized by the presence of a spatula shaped cap called pileus. This is fleshy part. Fruiting body of oyster mushroom has a stalk, which may be short, long, lateral or central. This stalk is called stipe. An interesting and attractive feature of oyster mushroom is presence of long ridges and furrows underneath the pileus which is called gills or lamellae. The gills bear the germinating spores, which help in reproduction of oyster mushrooms.

[10] However, some mushrooms are not edible and may cause vomiting or stomach pain if eaten but edible mushroom is considered as one of the important food sources. As they are easily digestible, they provide a simple yet nourishing meal for proper growth and good health. Edible mushrooms also regarded as the meat of the vegetable world can give a variety of delicious meals with different dishes and can also be used for adding flavors.[11] Mushrooms contain many nutrients, including fiber, B vitamins, phosphorus, vitamin D, selenium, copper, and potassium. They are also low in calories and fat. Report of protein and fat estimation as shown in Fig. 1. Mushrooms are also a good source of antioxidants, such as vitamin C, selenium, glutathione, and choline can help fight inflammation and oxidative stress.[12] Mushrooms have been not only used as food materials with their unique flavor and texture but also recognized as an important source of biologically active compounds of medicinal value.[13] Oyster mushrooms are also used in traditional medicine to treat high cholesterol, infections, diabetes, and cancer. Lab experiments suggest cholesterol-lowering, antifungal, and antitumor properties. Oyster mushrooms are also low in calories and help lose weight. They are a good choice for people on a low carb diet regimen, as they are high on protein and low in carbs. They are also suitable for people with hyper-tension, obesity and diabetes due to their low sodium: potassium ratio, starch, fat and calorific value.[14] Oyster mushrooms are highly nutritious and may promote heart and immune system health, encourage healthy blood sugar control, and provide antioxidant and anti-inflammatory effects. They can be used in a variety of dishes, including burgers, pizza, roasts, pasta, stews, and omelets.[14] Bakery product such as bread, cookies, biscuits and cakes are a large family of popular food products. This bakery products are consumed by a wide range of people. So we used mushroom (oyster) powder in bakery product like cookies, because of mushroom powder contain vitamins, minerals, protein, poly-phenols and fibers which are rich in source. Mushrooms are very perishable. Therefore, they should be consumed or processed promptly after harvesting. Drying is one of the significant preservation methods employed for storage of mushrooms and dried mushrooms are valuable ingredients in a variety of bread, cakes, biscuit, sauces and soups.[15] As mushrooms are very sensitive to temperature, choosing the correct drying technique can be the key to a successful operation. Many studies were done to drying of mushroom by different drying methods such as hot air, vacuum heat pump, freeze-drying, infrared (IR)-vacuum, osmotic dehydration , fluidized bed , IR and microwave. The sun-dried method was used to dry the mushrooms.[15] Mushroom powder is a rich source of nutraceuticals that are responsible for their antioxidant, antitumor and antimicrobial properties.[16] Medicinal mushroom extracts were considered as important remedies for the prevention of many diseases for thousands of years especially in the oriental regions such as cancer, diabetes, heart diseases, atherosclerosis, and cirrhosis.[16] Mushroom cookies is flavored due to its delicious flavor and low calorific content. Also they contain a high content of protein, fiber and other essential nutrients. There are number of health benefits of mushroom cookies such as Mushroom cookies are rich in nutrients like protein, fiber, minerals, vitamins B like riboflavin and niacin which help maintain energy levels. Vitamin D is essential for bone health, nerve function, and protecting skin from sun damage. They are great source of selenium, which is essential for health bones, teeth, hair, and nails, Mushrooms are low in calories and have 90% water content, making them excellent for weight loss and they are also gluten –free, have slow-releasing carbohydrates, and high in antioxidant.[17] The objectives are to cultivate mushrooms at room temperature and provide basic cultivation knowledge, prepare mushroom powder with high nutritional value, create ready-to-eat mushroom cookies that offer nutritional benefits and health advantages, and enhance the shelf life of both mushroom powder and cookies.

MATERIAL AND METHODS

Cultivation of Oyster Mushrooms

Oyster mushrooms were grown on various substrates such as paddy straw, wheat straw, rice straw, and vegetable plant residue. Wheat and rice straw were easily available and cheap, and they were widely used. About 500 g of wheat straw and rice straw were used, which were fresh and well-dried (Fig. 2). Wheat straw and rice straw were chopped into small pieces (Fig. 3). They were soaked in 25 L of fresh water for 9 to 10 hours. The excess water from the straw was drained by spreading it on filter paper or water-soaking paper. When the excess water from the straw was completely removed, it was ready for filling and spawning. Polythene bags were used for its cultivation. Spawning was done through layer spawning or through spawning (Fig. 4). In the case of layer spawning, substrate and seeds were filled in the bag and pressed to a depth of 8 to 10 cm. The second and third layers of substrate were put simultaneously after spawning. After 20 to 22 days, the bags were fully impregnated with white mycelium, and they were transferred into the cropping room, and polythene covers were removed. For mushroom cultivation, certain conditions were followed: mushrooms were grown in a temperature range of 20 to 30°C, relative humidity was maintained by spraying water twice a day on the walls and floor of the room, and a light spray was applied to holes as soon as small pinheads appeared (Fig. 5). Mushrooms were plucked before they shed spores to maintain quality. After the first harvest, 0.5 to 1 cm of the outer layer of the block was scraped off.

Preparation of Mushroom Powder

The mushrooms were dried out. It usually takes two days or so to dry the mushroom. The mushrooms were then ground into a fine powder in a spice grinder or blender, and any lumps were eliminated by straining the powder through a fine mesh sieve. The mushroom powder was kept cold in a tight jar.

Reparation of Mushroom Cookies

To prepare ready-to-eat mushroom cookies from mushroom powder, the following steps were followed: First, composite flour was prepared by mixing 15 g of all-purpose flour with 10 g of cocoa powder. Oyster mushroom powder, totaling 10 g, was added to the mixture (Fig. 6). Butter and ground sugar, amounting to 10 g, were premixed together, and vanilla extract was incorporated into this premix. The prepared composite flour was then added to the premix batter. A pinch of salt and baking powder were also added to enhance flavor and texture. The mixture was molded into cookie shapes. The air fryer was preheated for 5 to 7 minutes. The cookies were baked at 130°C for 15 to 17 minutes. Finally, the cookies were stored at ambient temperature for later consumption.

To study nutritional quality of mushroom cookies we conduct test such as

Moisture estimation of mushroom, mushroom powder and mushroom cookies[18]

The petri dish was placed in a hot air oven. After 30 minutes, the hot dish was transferred to a desiccator and allowed to cool. The initial weight of the petri dish was taken. The lid was removed, and the weight balance was tared. The sample was spread uniformly on the dish. The dish was closed and placed back in the hot air oven using the desiccator for transfer. The petri dish was kept in the hot air oven for 30 to 35 minutes. Afterward, the petri dish with the lid was removed and transferred to the desiccator to cool. The weight was measured, and the dish was returned to the oven for another 30 minutes, repeating the procedure. Three constant weights were obtained using the above method. Moisture content was calculated using the formula:

Moisture%= [(Initial weight of empty petri dish + Sample weight) – (Weight of final dish after drying sample)] / Sample weight

Ash estimation of mushroom, mushroom powder and mushroom cookies[19]

The initial weight of the dry and clean empty crucible was taken. The balance was tared, and 3 to 3.5 g of sample was weighed and placed into the crucible. The crucible was placed in the muffle furnace, using tongs and other protective accessories due to the warmth of the furnace. The sample was ignited for approximately 30 to 45 minutes at a specific temperature as required for the particular food. After 45 minutes, the muffle furnace was turned off, and the crucible was left to cool until the temperature dropped to 25°C or lower. The furnace door was opened carefully to prevent the loss of any fluffy ash, using tongs to quickly transfer the crucible to a desiccator. The desiccator was covered, and the crucible was allowed to cool (Figs 7 and 8). The weight of the crucible was measured, and ash content was calculated using the formula:

Ash%= [(Final weight of crucible – Initial weight of crucible) / Sample weight] x 100

Protein and fat estimation of mushroom cookies[20,21]

To estimate the protein content in mushroom cookies, 0.4 g of sample was taken and mixed with a 1:5 catalyst mixture of $CuSO_4$:K_2SO_4 and 10 mL of concentrated H_2SO_4. The mixture was heated and digested until the solution became clear, maintaining a temperature of 42°C. After cooling, 30 mL of distilled water was added to the distillation tubes. The solution was transferred to a titration flask, where 25 mL of 4% boric acid and methyl red indicator were added. The titration flask was allowed to cool. The titration was completed using standard HCl, and the percentage of protein was calculated using the formula. A testing certificate for protein and fat estimation was also attached.

Nitrogen%= [1.401 x normality of 1.0 N HCl x (BR– Blank)] Sample weight

Protein% = % nitrogen x conversion factor

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Test Report

Name of Customer	: Shingade Satyawan Namdev		
Address of the Customer	: Indiranagar, Chinchwad, Pune		
Date of sample Receipt	: 29/04/2024	Report Date	: 03/05/2024
Sample Code	: BSB/1122/F/CH/24		
Name of Sample	: Cookies	Condition of Sample	: Sealed (RT)
Quantity of Sample received	: 10 g	Sample Received From	: Mr. Satyawan Shingade
Discipline	: Chemical	Group	: Food and Agricultural product
Analysis start Date	: 30/04/2024	Analysis end date	: 03/05/2024
Testing Carried out at	: Permanent facility	Sample Collection Time	: 04.00 pm

Result Table

Sr. No.	Parameter	per serve (100 gm)	% contribution of RDA per serv	Reference Method
1	Protein	8.13%	%	Manual of Methods of Analysis o Foods- Cereal & Cereal Products, F 03.017: 2023
2	Fat	14.33%	%	IS 12711: 1989

----------End of Report ----------

Verified &Authorized by
(Chemical)
Mr. Vyankatesh Paatil

BIOSOURCE BIOTECH BSB PUNE 52

Note: 1. The above results pertain only to the sample tested & without prejudice of source/process
2. Any correction to this report invalidates the report.
3. This report can not be used for court purpose. We are not responsible for any legal matter.

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Fig. 1: Report of protein and fat estimation

Crude fiber of mushroom cookies[22]

To estimate crude fiber content present in Mushroom cookies, 2 to 2.5 g of sample was taken in a flask. Then, 200 mL of dilute H_2SO_4 was added. It was boiled for at least 20 to 30 minutes. The content in the flask was filtered through muslin cloth. The residue was washed with boiling water until it was no longer acidic. The neutral residue was carefully transferred to the flask and 200 mL of dilute NaOH solution was added. It was boiled again for 20 to 30 minutes. The content was filtered through the same muslin cloth that was previously used. The residue was washed with boiling distilled water until it was no longer basic. The residue was washed again, first with hot distilled water and then with 50 mL of 95% ethanol. The washed residue was transferred into a crucible and dried at 105°C. The crucible was ignited in a muffle furnace at 620°C until the residue was completely burned (Figs 9 and 10). The crucible containing ash was cooled and the final weight of the crucible with residue was taken. The estimate of crude fiber was calculated using the formula.

Crude fiber = [(Initial weight of crucible + Residue before ashing) – (Final weight of crucible + residue after ashing)]/Sample weight

Carbohydrate and energy value of mushroom cookies[23]

To estimate the carbohydrate content of mushroom cookies, the values of moisture, ash, fat, protein, and crude fiber were obtained through appropriate analyses following standard operating procedures (SOP) or test methods.

Eq. 6- Carbohydrate = 100- (% moisture+ %ash + %fat + %protein + % crude fiber)

To calculate the energy value of mushroom cookies, the nutritive values such as moisture, ash, protein, fat, and carbohydrate content were determined using methods recommended by the National Institute of Nutrition.

Eq. 7 Energy value = (carbohydrate + protein) x 4 + (fat x 9)

To study microbial quality of mushroom cookies we conduct test such as[24]

Total plate count

To check the total colonies on the given media, 1 g of sample was added to 10 mL of distilled water to create a dilution. Nutrient agar was then poured into petri dishes and allowed to solidify for approximately 10 minutes. Subsequently, 0.1 mL of the prepared sample was added to the center of each agar plate and spread evenly using a sterile spreader. The plates were then incubated at 37°C for 72 hours to allow bacterial growth. After incubation, the colonies were counted using a colony counter or by manually counting visible colonies on the agar plates. The number of colonies was calculated using the formula appropriate for the dilution factor applied during the sample preparation and plating process.

Colony forming unit/gm = Number of colonies on plate/ (volume of sample x dilution)

Fungal test

To check fungal growth on the given media, 1 g of sample was added to 10 mL of distilled water to create a dilution. Sabouraud's dextrose agar was then poured into petri dishes and allowed to solidify for approximately 10 minutes. Next, 0.1 mL of the prepared sample was inoculated onto the center of each agar plate and spread evenly using a sterile spreader. The plates were then incubated at 37°C for 72 hours to facilitate fungal growth. After incubation, the fungal colonies were counted using a colony counter or by manually counting visible colonies on the agar plates. The number of colonies was calculated based on the dilution factor used during sample preparation and plating.

Colony forming unit/gm = Number of colonies on plate/ (volume of sample x dilution)

RESULTS AND DISCUSSION

Mushroom cultivation using agro-waste materials like rice bran and wheat bran has been successful in lab conditions, yielding 200 and 500 g of oyster mushrooms, respectively, at room temperature. These mushrooms are then processed into mushroom powder, ensuring a ready-to-eat product that not only maintains a desirable taste and flavor but also offers health benefits. The process of growing mushrooms for cookies involves several steps: chopping rice straw, spawning with mushroom spawn, incubating the mixture, facilitating pin head formation, inducing sporulation, and finally harvesting mature mushrooms. These harvested mushrooms are subsequently dried, crushed into powder, and incorporated into cookie recipes before baking. This comprehensive process ensures that the mushroom cookies deliver both nutritional value and a distinctive mushroom flavor to consumers.

Table 1 presents the nutritional analysis of the mushroom cookies made using mushroom powder from cultivated oyster mushrooms and cocoa powder. Several tests were conducted to assess the nutritional content, revealing moisture content of 3.6%, ash content of 15.7%, energy value of 326.38 Kcal, protein content of 8.13%, total fat content of 14.33%, carbohydrates comprising 75.07%, and crude fiber content of 1.08%.

Fig. 2: Rice straw

Fig. 3: Chopped raw material

Fig. 4: Spawning

Fig. 5: Pin head

Table 2 indicates that the moisture content of dried mushrooms is 2%, which is lower than that of fresh mushrooms. This characteristic

Fig. 6: Oyster mushroom

Fig. 7: Dry mushroom

Fig. 8: Mushroom powder

Fig. 9: Cookies batter

aids in controlling microbial growth and contributes to extending the shelf life of the product. Additionally, dried mushrooms contain 5.19% ash.

Based on FSSAI guidelines, the total plate count (TPC) limit for cookies and other bakery products is typically less than 10^5 cfu/g (i.e., fewer than 100,000 colony-forming units per gram), and the fungal count limit is generally less than 10^3 cfu/g (i.e., fewer than

Fig. 10: Mushroom cookies

Table 1: Nutritional value of mushroom cookies

Test performed	*Result (%)*
Moisture test	3.6
Ash test	15.7
Protein test	8.13
Carbohydrate test	75.07
Fat test	14.33
Crude fiber	1.08
Energy	326.38 Kcal

Table 2: Test performed for dry mushroom

Test performed	*Result (%)*
Moisture test	2
Ash test	5.19

Table 3: Microbial test performed for mushroom cookies

Microbial test	*Total plate count (CFU/gm)*	*Fungi and mould (CFU/mL)*
Day 1	0	1×10^1
Day 15	0.9×10^{-3}	2×10^1
Day 25	2.1×10^{-3}	6×10^1
Day 35	3.5×10^{-3}	13×10^1

1,000 colony-forming units per gram) (Table 3). Our study of the microbial quality of mushroom cookies, as referenced from the FSSAI guidelines on their official website (https://fssai.gov.in/), indicates that the microbial quality of these cookies is within acceptable limits.

CONCLUSION

Recent years have seen higher incomes and more active lifestyles globally, leading consumers to seek high-quality convenience foods. Bakery items such as cookies, bread, and rolls are enjoyed worldwide. This study aimed to cultivate mushrooms and prepare cookies using mushroom powder and locally available raw materials to enhance their quality. The objective was to develop a standardized methodology for producing mushroom cookies. Mushrooms were sourced from the Kulkarni mushroom cultivation farm and analyzed for moisture content, revealing that mushrooms are rich in nutrients, protein, minerals, and dietary fiber. Selected oyster mushrooms were sun-

dried until the moisture content reached 2%, then processed into powder form to reduce moisture content, increase nutritional value, and extend the product's shelf life. The resulting cookies, made with mushroom powder and cocoa powder, offer a nutritious option for daily consumption. The mushroom cookies contain 3.6% moisture, 15.7% ash, 8.13% protein, 75.07% carbohydrates, 14.33% fat, 1.08% crude fiber, and provide 326.38 kcal of energy. In conclusion, the cultivation and characterization of oyster mushrooms and their application in confectioneries were successfully accomplished.

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