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Efficacy of *Pleurotus eryngii* Mycelium Containers as an Alternative to Current Single-Use Plastic Based Methods

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Abstract
Introduction: The COVID-19 pandemic has led to an increase in takeout orders due to the mandated loss of dine-in options at restaurants. The significant rise in takeout has led to the use of single-use plastic containers that are currently made from materials such as polyethylene and expanded polystyrene (EPS). The aim of our research is to determine the effectiveness of *Pleurotus eryngii* (King oyster mushroom) based mycelium in replacing single-use takeout food containers.
Methods: Variables such as flexural strength, permeability, insulation test, interactions between food and mycelium container, and decomposability in comparison to the common EPS takeout container. Various articles from scholarly sources such as PubMed, Google Scholar, and Omni library were used to determine the anticipated results.
Results (hypothesized): The flexural strength test will be greater than or equal to 0.43 mPa. The mycelium container should be able to stop various viscosities of food from diffusing through the container due to the hydrophobicity of the mycelia. The mycelium should have greater insulation capacity than the standard EPS container. Qualitative observations obtained for interactions between the mycelium container and food should be similar to the control EPS container.
Discussion: The mycelium container will be an effective alternative to using single-use EPS containers due to all tested variables resulting in the same or greater capabilities than the EPS container.
Conclusion: Overall, using mycelium containers as an alternative to single use plastic takeout containers would reduce plastic waste and emission pollution, having a positive impact on climate change.

Keywords: mycelium; polyethylene; expanded polystyrene; single-use plastics; food containers; greenhouse gases; methane; *Pleurotus eryngii*; plastic pollution

Introduction

In the past 20 years, greenhouse gas emissions (GGE) have increased by 45% due to a large contribution of plastic waste [1]. In 2015, plastic waste incineration gave rise to six million metric tonnes of CO_2 [1], constituting 36% of global GGE [2]. These emissions are said to triple by 2030 if incineration rates continue to rise as they are. However, methane's ability to trap heat is twenty-eight times greater than CO_2, in which the decomposition of plastics contributes to 18% of global methane pollution [3]. Plastic pollutants, such as single-use plastics, are non-biodegradable and account for half of human-made waste [4] found in oceans as well as 86% of landfill waste in Canada [5]. The impact of single-use plastics has dramatically increased as a result of the COVID-19 pandemic through the constant use of medical testing kits, disposable masks, and personal protection equipment [6]. In a study by Ana L. Patricio, it is reported that in Catalonia, Spain, and in China there was an increase of 350% and 370% respectively in personal protective equipment waste. [7]

Another aspect of single-use plastics is seen in the restaurant industry. Since the beginning of the COVID-19 pandemic, the lack of indoor dining has caused catering enterprises to increase online food ordering operations. The rapid development of the food ordering industry, hence the takeout food industry [8], led restaurants to utilize single-use plastics due to their cost-effectiveness and accessibility. However, an analysis of 12 million items from seven major aquatic environments found that 80% of items were plastic, and nearly half were related to takeout food and drinks [4]. This study demonstrates the prevalence of single-use plastics found in natural ecosystems, which may coincidentally have dangerous implications towards the ecosystem chain as well as human health in the long run.

Commonly found in the takeout industry, single-use containers are typically made from materials like polyethylene and expanded polystyre (EPS) [9]. Polyethylene can increase the number of endocrine disruptors in the human body, mimicking estrogen which overstimulates cancerous cell growth and decreases human fertility [10]. Meanwhile, microplastic pollution of polystyrene is known to cause neurological damage, impacting learning and memory behavior [11], as well as cytogenetic and DNA damage after continuous exposure, hence increasing risk of cancer [12].

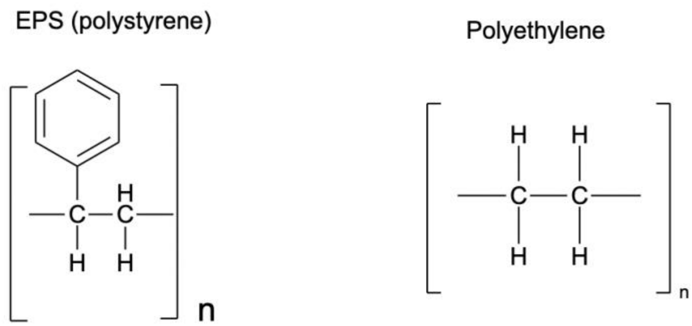

Figure 1. Chemical structure of expanded polystyrene (left) and polyethylene (right). Created using ChemDraw.

Although recycling is encouraged, producing new plastic products is cost-effective and easily manufacturable. As a result, 2% of plastic waste is recycled into a product of the same value and 8% [4] is recycled into a product of lower value, while the remaining 90% is incinerated or landfilled. Greenhouse gas emissions and climate change is expected to drive disease migration, extreme weather, and increased effects of air pollutants on global health [13].

There are many biodegradable alternatives to polyethylene and polystyrene, the most promising being mycelium, the root-like structure of fungi. Previous studies have used mycelium to create packaging and construction materials, such as bricks and cement. In Xing et. al.'s study, mycelium bricks were grown and tested as building insulation materials. This bio-based material demonstrated similar physical and mechanical properties to existing materials, such as expanded polystyrene, and an enhanced level of biodegradability and good thermal performance [14].

These findings show mycelium's promising ability to bind to organic matter through a network of hyphal microfilaments on an industrial scale with minimal water and light and a quick growth rate. Furthermore, it is found that hyphae microfilament binding allows mycelium-based materials to have hydrophobic properties [15], stability at high temperatures, and a low density of 0.10 to 0.39g/cm [16]. Despite its low density, mycelium may demonstrate a flexural strength up to 72 MPa [17]. Although mycelium is a biological material, it is stronger than polystyrene foam products and has a shorter lifespan [16]. Mycelium food containers are also not expected to produce by-products as these organic products decompose within a month [18].

Using previous findings, the aim of our research is to determine the effectiveness of *Pleurotus eryngii* based mycelium in replacing single-use takeout containers to sustainably reduce global greenhouse gas emissions and plastic pollution.

If mycelium replaces single-use plastic containers, the net plastic waste in oceans and landfills would dramatically decline. With mycelium-based containers, the amount of plastic waste in the ocean could decline by at least 9% [4]. The amount of atmospheric methane will consequently decrease after eliminating major plastic pollutants. Mycelium-based food containers can be used to upcycle agricultural wastes and decrease the carbon/methane footprint.

With proper testing, mycelium may prove to be a viable alternative to single-use plastic food containers, thus reducing global greenhouse gas emissions and plastic pollution due to its accessibility, biodegradability, and durability.

Methods

In a controlled laboratory setting for 5 weeks, we hypothesize a method of various tests to assess the durability and the efficacy of a *Pleurotus eryngii* mycelium-based takeout container when exposed to various types of food. Currently existing EPS containers will be used as a control. After its production phase, the mycelium containers will be tested for flexural strength, permeability, thermal insulation, and interactions between food and the container. A total of 250 mycelium containers are compared with 250 EPS containers.

Replicated *Pleurotus eryngii* mycelium vegetative tissue is introduced to a filter patch bag and grows with sufficient moisture, energy, and nutrients; calcium, nitrogen, and carbohydrates. The *Pleurotus eryngii* substrate is composed of hardwood sawdust [19] from environmental waste. The substrate is sterilized with heat and pressure before inoculation, ensuring minimal contamination. The first inoculation step is expected to take two weeks [19]. The enclosed laboratory conditions provide minimal light with a temperature between 25ºC to 35ºC [20]. The inoculation process would be visible within the first week and the hyphae develop throughout the second week.

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Table 1. Different parts of the methodology and the needed number of containers for each part. Created using Google Docs.

Experiment Part	Description	Number of Containers Being Tested
A	Production of Mycelium Containers	N/A
B	Flexural Strength Test	50 mycelium 50 EPS
C	Permeability of Mycelium	50 mycelium 50 EPS
D	Insulation Test	150 mycelium 150 EPS
E	Testing Interactions Between Food and Mycelium Container as well as Decomposability	Same containers used in part D
F	Flexural Strength After Food	Same containers used in part D

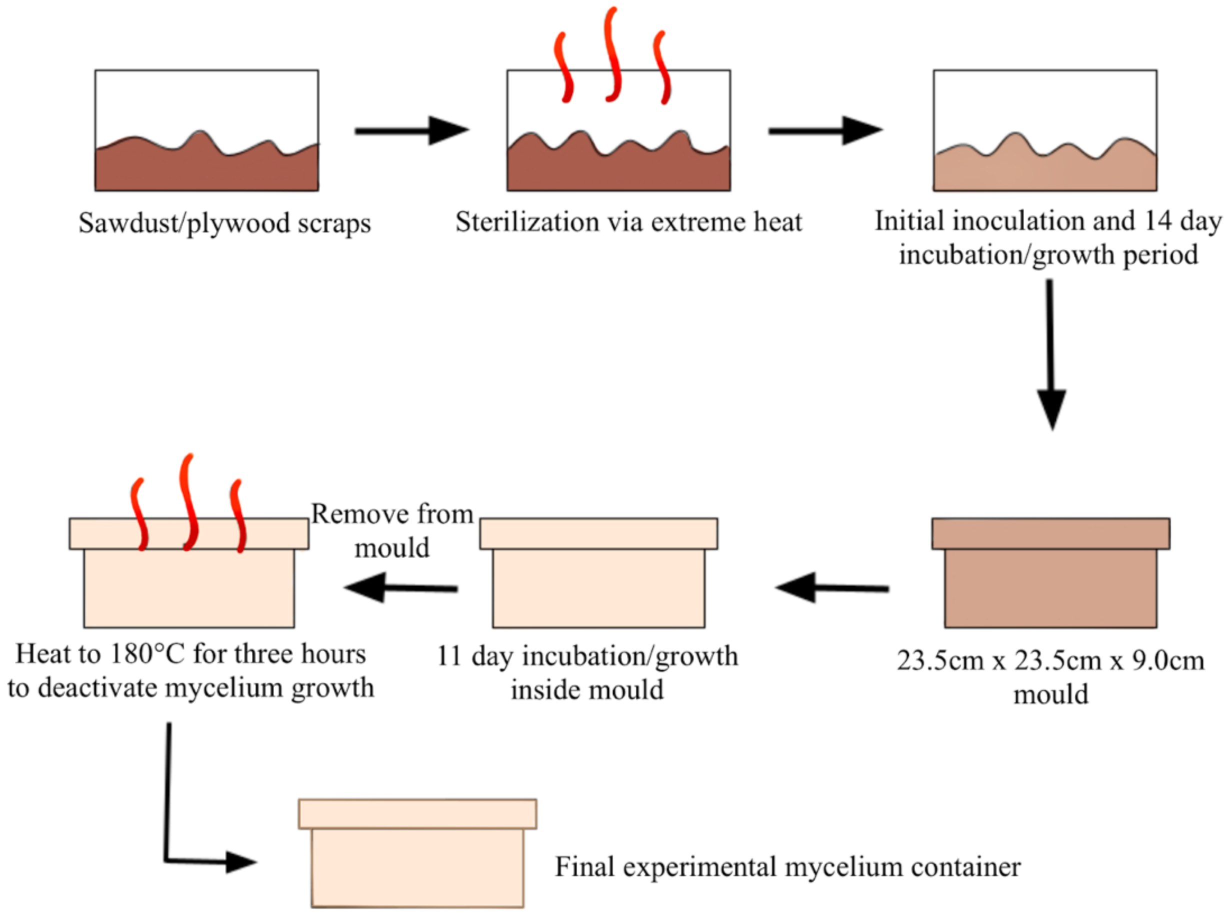

Figure 2. General process of how mycelium is grown with substrate, deactivated, and formed into a final experimental container. Created using Procreate®.

The inoculated substrate is divided into ten pieces to homogenize mycelium growth and ensure uniform density. The material is packed into tile moulds (Figure 2) to imitate a common takeout container. Tissue growth should be seen on the surface of the samples within eleven days [20].

Sheets of fungal biomass are removed from the mold and dried [20] at 180°C for three hours to ensure a dry mycelium sample. This slow low-level baking deactivates the hyphae and halts the growth process [21].

To test flexibility, the experimental *Pleurotus eryngii* mycelium container is cut into 20mm×20mm×15mm specimens for mechanical testing to analyze the durability of the mycelium container after exposure to food. A flexural strength test is applied to analyze the maximum bending properties of materials prior to cracking.

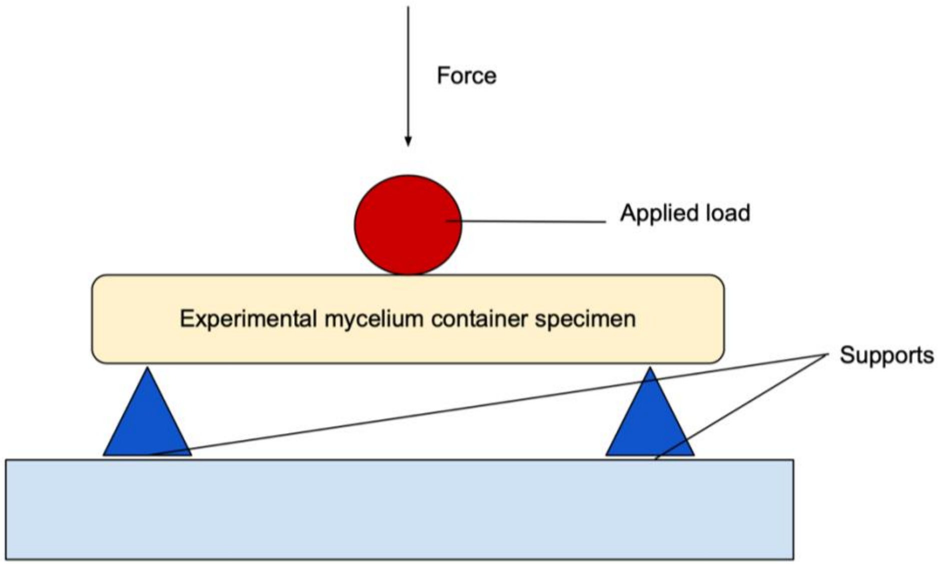

Figure 3. 3-point flexural strength test on the experimental mycelium container specimen. Created using Google Drawings.

A flexural strength equal to or greater than 0.43 mPa [17] indicates success of the experiment, suggesting that the mycelium container demonstrates a similar flexural strength to an EPS container. Based on an overview of materials for 146 pieces of expanded polystyrene, 0.43 mPa is the average flexural yield strength value. Based on a previous study by Appels et. al., heat-pressed *Pleurotus ostreatus*-cotton mycelium material demonstrated a flexural strength of 0.62 ± 0.11 mPa. It is expected for our experimental *Pleurotus eryngii* to exhibit a similar value in flexural strength [16].

To test the effectiveness of the mycelium product, various foods are introduced to mycelium containers and compared to the control group, expanded polystyrene foam (EPS) containers, in acidic, neutral, and basic conditions for four days. Four days is the length in which leftovers remain safe to eat when stored safely in the refrigerator. Surpassing four days, the leftovers are likely to spoil or become a health risk due to bacterial growth [22]. Teriyaki noodles, cheeseburgers, and lemongrass salad are added, with pH 4.5-5 [23], 7.0 [24], and 8.0 [24] respectively. Darcy's Law of Permeability, as shown, describes the fluid flow through porous media while also relating the permeability of the medium, resulting in an accurate measurement of the permeability of our mycelium containers. To begin, canola oil is inserted into the fluid reservoir and pumped through a 1 mm thick mycelium sheet. The oil is pumped through using a vacuum pump and collected as produced fluid (Figure 4). The volume of the oil before and after are recorded and compared to the control group to determine the permeability of the mycelium container. This experiment is run through 50 mycelium containers and 50 EPS containers.

To examine the interactions between the mycelium containers and food, an analysis with various foods at different pH ranging from acidic, neutral, to basic will be conducted using teriyaki noodles, cheeseburgers, and lemongrass salad respectively.

A sample size of 50 containers per food type is implemented for mycelium and EPS; they will be subjected to thermal insulation and interaction tests, which will be done in succession.

The thermal insulation of the container is tested through a longitudinal study, eventually determining the heat capacity of the container. To perform this study, the internal temperature of the food is checked every 30 minutes using a thermometer and compared to the control group for a total of 2 hours. Afterwards, the containers are put into a controlled fridge environment at 3°C for 4 days. This timeline was chosen to mimic routine-like conditions for a typical takeout food order.

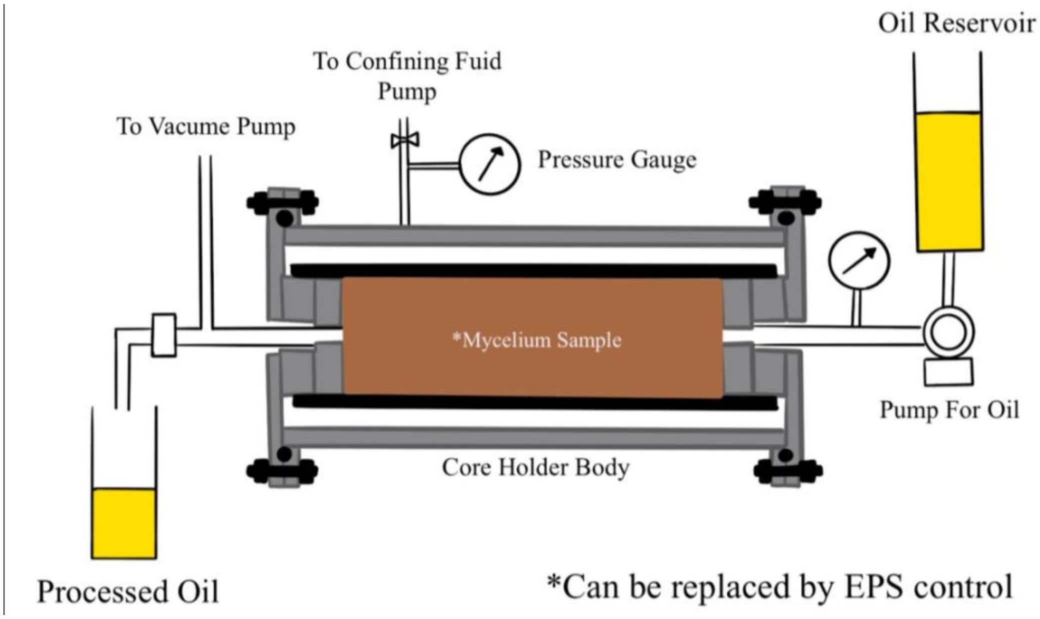

Figure 4. Oil permeability test set up used to find and calculate the permeability of the mycelium containers. Created using Procreate®.

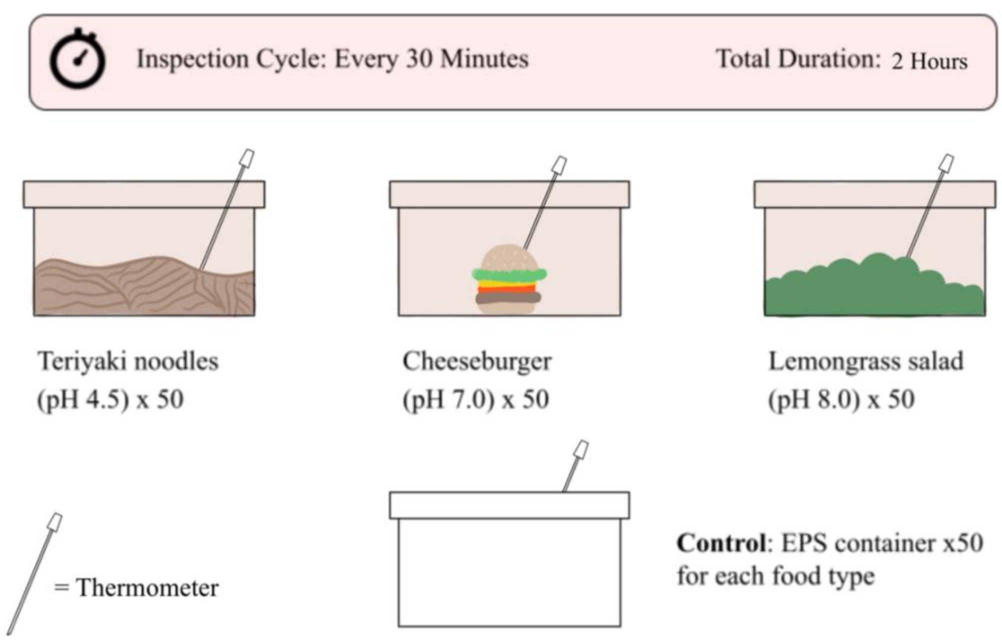

Figure 5. Simple set-up of the thermal insulation test for the mycelium containers containing various foods (teriyaki noodles, cheeseburger, and lemongrass salad) and its duration/inspection cycle. Created using Procreate® and Google Drawings.

Testing the interactions between food variables and containers is done to observe the visible qualitative change in the food stored. This is done by 12-hour check-ups throughout the course of the 4-day long study. The main deterring point would be visible alterations on the food, as risk of food poisoning increases after 3-4 days. The expected qualitative results would be similar to EPS containers.

After 4 days, another flexibility test will be performed on the soiled containers. This is to ensure that the containers maintain their initial flexural strength despite being exposed to non-ideal conditions. Following this final test, the containers will be composted, and the time of decomposition will be taken. It is expected that the containers will decompose in 6 weeks.

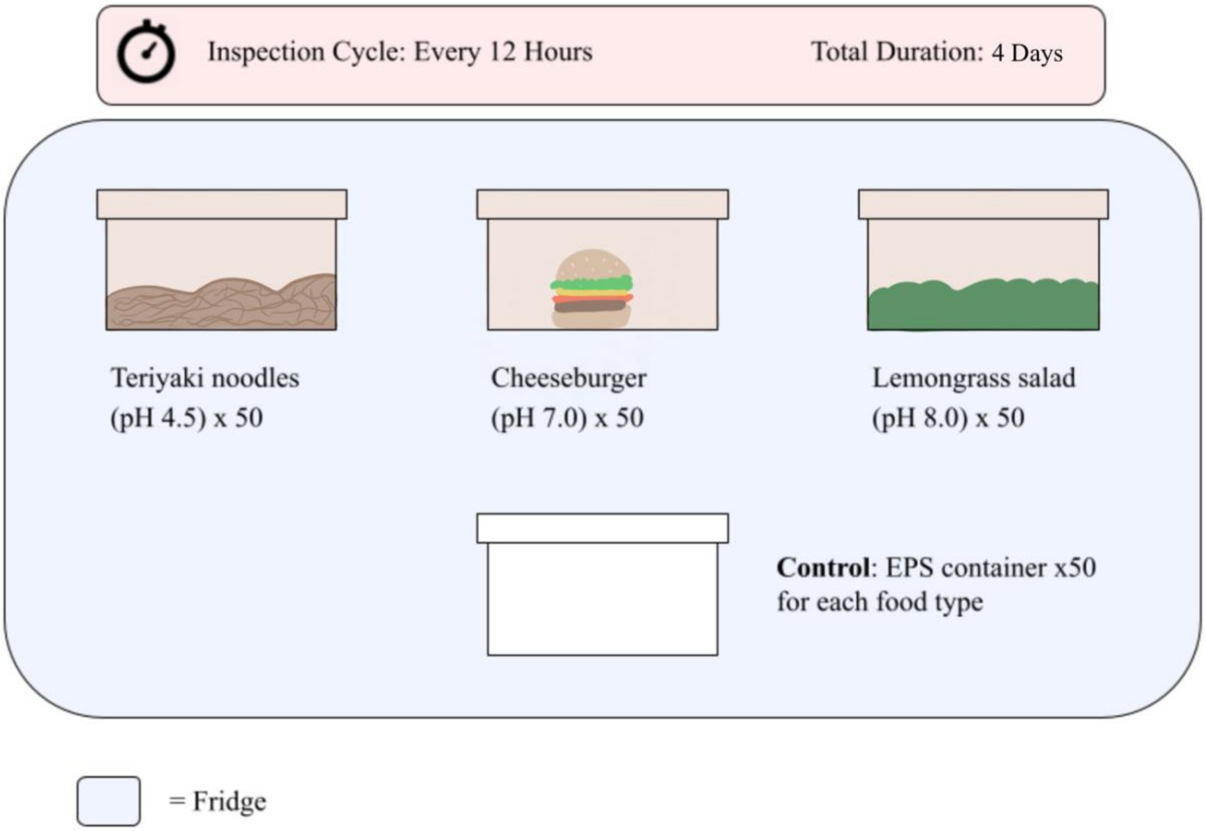

Figure 6. Set up for the testing of interactions between the mycelium containers and different foods at various pH. Created using Procreate® and Google Drawings.

Results

After the production of the *Pleurotus eryngii* mycelium containers, it is hypothesized that Part B of the methodology will result equal to or greater than 0.43 mPa during the flexural strength test. This value would indicate success of the experiment, suggesting that the mycelium container demonstrates a similar flexural strength to an EPS container.

The permeability test in Part C would reveal that the *Pleurotus eryngii* container is able to maintain the volume of canola oil pumped through the container just as well as the EPS control group. This is due to its hydrophobic properties. Viscous foods, such as the teriyaki noodles and lemongrass salad, would not affect the container as the mycelium in the container has also been deactivated through the baking process to ensure there is no reaction. Chemical reactivity between the experimental mycelium container and the food materials are not likely to occur due to the stable chemical composition of mycelium. Mycelium is mainly composed of polymers, such as chitin, cellulose, and protein [26], which demonstrate low chemical reactivity and low solubility [27]. As a result, it is not expected for the tested *Pleurotus eryngii* containers to affect the food materials in a toxic or otherwise undesirable manner. These results are desirable because the mycelium container would demonstrate similar results to the EPS container.

The insulation tests on *Pleurotus eryngii* container during Part D would result in a greater insulation capacity as well as no accidental carcinogens in your food. Through previous studies [28] conducted, mycelium has been proven to be a viable eco-friendly alternative to current insulation methods without the use of carcinogenic materials. With a low value of thermal conductivity (0.024W/m.K.), mycelium becomes a more efficient insulator [29] when compared to polystyrene (0.036W/m.K).

When testing the interactions between the *Pleurotus eryngii* container and food during Part E, it is expected to see qualitative results similar to the control EPS containers. Risk of food poisoning increases after 3-4 days [30] because of food spoilage. Hence, alterations in odor and appearance of the food, which are characteristic of signs for food spoilage, are deterring points for this experiment.

After performing the final flexural strength test on the soiled containers in Part F, it is expected to obtain a strength of around 0.43 mPa. This is due to *Pleurotus eryngii* mycelium's hydrophobic properties and dense hyphal webbing that would prevent viscosities of food to penetrate through and weaken the material, allowing it to maintain its structural integrity.

Discussion

These expected results show that the experimental *Pleurotus eryngii*-based mycelium container demonstrates similar or greater efficacy to existing EPS plastic containers under multiple tested conditions. Our findings concerning the mycelium container's flexibility, permeability, insulation, and interactions with food extend the work of bio-based insulation building materials [31]. With our method, we observe the use of this novel type of bio-insulation material being used as a sustainable and biodegradable alternative to single-use plastic containers. The results show that mycelium containers demonstrate similar physical and mechanical properties to existing single-use plastics, such as expanded polystyrene, however, exhibit an enhanced biodegradability. As our method demonstrates that mycelium containers show similar usefulness, the container demonstrates to be a viable and desirable alternative to potential takeout food customers.

However, we note that future studies are required to thoroughly compare the interactions between food and the mycelium container to ensure food safety. Concerning the FDA, takeout containers are considered "housewares" and are usually except from premarket clearance requirements. However, mycelium may be considered a new food contact substance and require further regulation. According to Title 21 of the Code of Federal Regulation, Part 170.39 "Threshold of regulation for substances used in food-contact articles", a substance used in a food container such as mycelium that may have the potential to migrate can be exempt from regulations as a food additive if it is under the threshold for certain characteristics [22]. For example, the substance must not be carcinogenic and must not present health/safety concerns. As previously stated, mycelium will not show any carcinogenic properties and should not present any health concerns.

The thinly replicated mycelium tissue in this study may provide implications towards future alternatives for similar single-use plastics, such as plastic water bottles. By substituting harmful plastic materials, our method encourages the reduction of plastic and emission pollution.

Conclusions

The main purpose of this study is to determine the overall efficacy of mycelium, which demonstrates to be a sustainable and biodegradable replacement of single-use plastic takeout containers. The experimental mycelium container is expected to be similarly or exceedingly competent in comparison to current EPS containers. These mycelium containers provide a breakthrough for the food and take-out industry. As previously stated, plastic waste currently accounts for ½ of human-made waste in oceans [4] and 86% of landfill waste in Canada [5]. Our study proposes that the use of this bio-based material will decrease plastic waste in the environment and therefore reduce further GGE pollution. Additional research into mycelium and its properties can lead to innovative solutions to single-use plastics, including single-use cutlery and bottles. Mycelium provides a quick growth rate while feeding off of organic waste through its hydrophobic hyphae microfilaments. Due to these various properties, future studies are warranted to determine the efficacy of the mycelium container and explore future applications of this material.

List of Abbreviations Used

CO_2: carbon dioxide
COVID-19: coronavirus disease 2019
EPS: expanded polystyrene
GGE: greenhouse gas emission

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Approval and/or Participant Consent

This proposal did not require an ethics of approval as it was designed for a case competition.

Authors' Contributions

JGK: Made contributions to the development of the study, organization of methodology, analysis of expected results, and gave final approval of the version to be published.
SS: Made contributions to the design of the study, organization of methodology, analysis of expected results, and gave final approval of the version to be published.
SKC: Made contributions to the design of the study, collection of previous studies, collaborated further discussions, and gave final approval of the version to be published.
LE: Made contributions to references and formatting, collection of previous studies, and gave final approval of the version to be published.

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