ABSTRACT

PISTACHIO BYPRODUCTS AS SUBSTRATE FOR SHIITAKE MUSHROOMS

The United States is expected to lead the global pistachio industry during the 2009-2010 harvest season, bringing in about 175,000 metric tons of nuts. Approximately 63% of this pistachio harvest will be composed of shells and hulls, the byproducts of the pistachio industry. The disposal of these byproducts is proving costly to producers, and many current disposal methods are damaging to the environment. It was the objective of this study to develop a potential market for pistachio shells and hulls by utilizing them as a mushroom substrate component. The production of shiitake mushrooms on a substrate composed of pistachio shells and hulls was evaluated to determine their suitability for this use.

Five experimental substrates were developed using different ratios of shells and hulls. The mushroom production on some experimental blocks was shown to be more prolific than that of the control blocks, demonstrating that pistachio shells and hulls are a suitable substrate for the growth of shiitake mushrooms. Various quality parameters were measured on the harvested mushrooms such as color, texture, moisture content, and size, and it was found that the mushrooms grown on experimental substrates showed similar characteristics to those grown on the control. This study demonstrates potential to minimize or even eliminate the pistachio harvest waste stream, thus eliminating the environmental hazards associated with their current disposal, while at the same time developing a more cost effective substrate for the production of shiitake mushrooms.

Chelsea Anne Zweigle
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PISTACHIO BYPROUCTS AS SUBSTRATE FOR SHIITAKE MUSHROOMS

by

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APPROVED

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INTRODUCTION

Worldwide pistachio production during the 2009-2010 growing season is expected to reach 395,000 metric tons, with 175,000 metric tons expected to come from the United States (USDA-FAS 2010). This anticipated harvest will make the United States the frontrunner in worldwide pistachio production, and is a direct result of the significant increase in bearing acreage in the California pistachio industry. The reported weight of the pistachio harvest is not an accurate representation of the amount of material harvested from the tree, as it only represents the weight of the actual nut kernels and shells. The weight of the outer hull that encases the kernel and shell is neglected in these estimates, despite the fact that it represents a large portion of the actual harvest weight. The hulls, considered a byproduct of the pistachio harvest, are separated immediately after the nuts are removed from the trees, and spread on the orchard as mulch. Until recently, most pistachio nuts were sold in shell, however recent trends to use them as ingredients in value added products has forced processors to remove the shells prior to distribution, significantly increasing the amount of solid byproducts generated. No reliable means of using the hull or shell byproducts has been developed thus far, resulting in added costs for the pistachio industry, as they must devise ways of disposing of these byproducts. The labor and fuel required to spread hulls on the orchards and deposit the shells in landfills results in a significant amount of time and money lost by producers each year.

This study is an attempt to develop an alternative market for the pistachio industry byproducts, thus generating additional income for farmers, and reducing

the environmental hazards that their current disposal presents. Although there is limited information available on the composition of pistachio shells and hulls, their lignocellulose nature may prove useful to the mushroom industry for use as a substrate component. Shiitake mushrooms in particular, require a substrate composed of both lignin and cellulose materials for their successful cultivation, and it is thought that the combination of shells and hulls may create a viable nutrient source for their growth. It is the goal of this study to develop an alternative substrate for the cultivation of shiitake mushrooms, while at the same time utilizing currently unused byproducts of the pistachio industry. If shells and hulls prove as a viable ingredient in shiitake mushroom substrate, the benefits to the pistachio industry, shiitake mushroom industry, and the environment would be significant. Pistachio farmers would have the potential to earn more profit from their crop each year, and the environmental hazards caused by their current disposal could potentially be eliminated.

Goals and Objectives

The goal of this study was to develop an environmentally friendly utilization for pistachio processing byproducts. The specific objectives included determining if shiitake mushrooms would grow on a substrate composed of pistachio shells and hulls, and evaluating the overall quality of the resulting mushrooms in terms of moisture, color, texture, and size.

Chapter 2

LITERATURE REVIEW

Mushroom

The mushroom is the fruiting body of a complex network of fungal mycelia. The vegetative fungus that supports the mushroom branches out through a substrate such as soil, compost, wood, or a variety of alternative lignocellulosic materials. When the mycelium is fully developed, and the environmental conditions are right, fruiting bodies form for the purposes of sexual reproduction. The most common form of mushrooms is umbrella shaped and comprised of a cap and stem (pileus and stipe) (Chang 2008). The mushrooms act as a reproductive body by releasing spores from the gill area on the underside of the cap.

Mushrooms can be classified into three ecological categories: saprophytes, parasites, and mycorrhiza (Chang 2008). The majority of mushrooms cultivated for human consumption are considered saprophytes, meaning they derive their nutrients from dead organic materials (Chang 2008). Although some mycorrhiza mushrooms are edible, they are difficult to grow commercially, as they rely on a symbiotic relationship with host plants or animals for their survival that would not easily be replicated in captivity. The final category of mushrooms, parasitic, describes fungi that thrive from their relationship with other organisms, while at the same time deteriorating their host organism. Mushrooms are also classified into four groups: edible, medicinal, poisonous, and other (Chang 2008). Mushrooms that fit into the other category are mushrooms whose characteristics are not well known or are potentially undiscovered to date.

Unlike plants, mushrooms lack the ability to derive nutrients or energy from the sun, as they do not contain chlorophyll. Instead, they rely on lignocellulosic materials, which are comprised of cellulose, hemicelluloses, and lignin, to sustain them (Chang 2008). Agriculture and forestry wastes are excellent sources of lignocellulosic materials for mushroom cultivation. As these wastes have traditionally been burned, it has been suggested that mushroom cultivation could reduce environmental pollution, by eliminating the excess burning of forestry and agricultural waste, as well as provide a steady source of food for growing human populations.

Shiitake

Lentinula edodes is a variety of mushroom that is very popular in China (known as xiang gu in Chinese), Japan (shiitake in Japanese), and throughout Asia. Originally cultivated in China between A.D. 1000 and 1100, followed by Japan about 500 years later (Chang and Miles 2004), the mushroom has gained tremendous popularity for its nutritional and medicinal value. Since 2002, it has earned the title of the world's most cultivated mushroom species (Chang 2008). shiitake is a saprophytic mushroom that grows on woody substrates such as dead logs and stumps. It was not until the late 1950's that an alternative substrate and cultivation technique was developed in China that utilized an artificial substrate.

Although its roots lay in Asia, the shiitake has spread throughout the world. The development of alternative growing techniques has allowed the mushroom to be cultivated in foreign environments, including the United States. Although it is relatively new to the United States, the 2008-2009 growing season yielded a sales volume of 9.42 million pounds, with a price of $3.19 per pound achieved by the

growers (USDA-NASS 2009), up from the 1998-1999 growing season, during which 6.24 million pounds were sold for $3.09 per pound (USDA-NASS 1999).

Medicinal and Nutritional Qualities

Shiitake is one of the 700 species of mushrooms that are said to possess medicinal qualities. Biologically active polysaccharides are the compounds within many species of mushrooms that have medicinal characteristics. These active compounds can be extracted from the fruiting body, mycelium, or liquid cultivated broth (Chang and Miles 2004). Shiitake mushrooms are shown to have antiviral, antitumor, cardiovascular, and renal effects, as well as antioxidant activity (Chang and Miles 2004; Cheung 2008).

The water-soluble polysaccharide, Lentinan, found in shiitake has been approved for use on humans as an anti-cancer drug in Japan, as it has been shown to trigger both killer and helper "T" cells. The polysaccharide KS-2, also found in shiitake, has shown promising results in the reduction of carcinoma in mice (Stamets 1993).

In addition to their medicinal benefits, shiitake mushrooms provide an excellent source of nutrition. The protein found in mushrooms contains all nine essential amino acids (Table 1), with especially high levels of leucine and lysine, making shiitake mushrooms an important vegetarian source of these amino acids that are otherwise not present in cereal based foods (Chang 1980; Chang and Miles 2004). In addition to being composed of approximately 13-18% protein, 3.5-6.5% ash, 6-15% fiber, and 2-5% fat, shiitake mushrooms also contain a variety of vitamins and minerals that are essential to the human diet (Stamets 1993) (Table 2).

Table 1 – Amino acid profile of *Lentinula edodes* (g/100g protein)

	Amino Acid	Amount
Essential	Isoleucine	4.4
	Leucine	7.0
	Lysine	3.5
	Methionine	1.8
	Phenylalanine	5.3
	Threonine	5.2
	Valine	5.2
	Tryosine	5.2
	Tryptophan	ND*
Non Essential	Alanine	6.1
	Arginine	7.0
	Aspartic Acid	7.9
	Cystine	ND*
	Glutamic Acid	27.2
	Glycine	4.4
	Histidine	1.8
	Proline	4.4
	Serine	5.2

*Not Determined

Adapted from Chang 1980

Table 2 – Vitamin and mineral composition of *Lentinula edodes* (mg/100g)

Name	Fresh	Dry
Niacin	55.0[a]	11.9
Thiamin	7.8[a]	0.4
Riboflavin	5.0[a]	0.9
Ascorbic Acid	0	0
Calcium	98.0	118.0
Phosphorous	476.0	171.0
Iron	8.5	4.0
Potassium	ND*	380.0
Sodium	61.0	19.0
Magnesium	ND*	247.0[b]

*Not Determined
Source unless otherwise noted: Food and Agriculture Organization (1972)

[a]Source: Stamets (1993)

[b]Source: Adriano and Cruz (1933)

Factors Influencing the Growth of Shiitake

Many genotypes of shiitake mushrooms are used in commercial production. Scientific literature is lacking information on which of these genotypes grows best on artificial logs, and what specific growing conditions and substrates are preferred by them. There are few consistencies within the research in regards to growing shiitake mushrooms from start to finish. While most cultivators use a similar process, adjustments of varying degrees are made at each step to tailor the process to their own needs, making each process unique.

In order to objectively compare the conversion of substrate material to harvested mushrooms, growers rely on the calculation of the biological efficiency

(BE) of their blocks. The BE is determined by the weight of mushrooms harvested (wet weight basis) divided by the weight of the substrate (dry weight basis), multiplied by 100%. This simple equation provides an easy way to evaluate the success of various substrate materials or cultivation techniques.

The composition of the substrate is one area where growers are at liberty to use materials that suit their needs in the most cost effective way. The first decision that must be made in commercial shiitake production is if the mushrooms will be grown on artificial or natural logs. It has been demonstrated that there are many benefits to using synthetic logs during shiitake production, including a shorter span of time from inoculation to harvest, better yield, and year round availability, regardless of season (Royse and others 1989). It has also been demonstrated that BE as high as 145% have been obtained on artificial logs over a period of six months, while only 9-35% efficiencies should be expected from natural logs over a 6-year period of time (Royse 1985). With this in mind, focus will be drawn to the composition of artificial logs, and the subsequent effect on mushroom development.

Most synthetic substrates are composed of about 50% wood chips and 50% nutrient supplements. While sawdust is the most commonly used substrate for shiitake cultivation, other supplements are frequently added such as rice bran, wheat bran, millet, rye, and maize. In addition to these plant based additives, calcium carbonate can be added to enhance the nutritional value of the mushroom substrate. In one study (Royse and Sanchez-Vasquez 2003), the performance of logs supplemented with various levels of calcium carbonate was juxtaposed with the performance of logs that were not supplemented. The BE of blocks that were not supplemented with calcium carbonate was around 62.3%, while those that were supplemented with 0.4% calcium carbonate, reached 90.6% BE. Clearly the

addition of calcium carbonate had a positive impact on the mushroom production in this study.

Moisture content is also a very important factor in the growth of shiitake mushrooms. The most recurring substrate moisture content is roughly 60% (Royse 2001; Royse and Sanchez-Vasquez 2001, 2003; Philippoussis and others 2007; Shen and others 2008), however a study by Badham (1989) considered a variety of moisture contents and their influence on the growth of Shiitake. Moisture contents ranged from 24-57%, and it was determined that the shiitake mycelium grew most rapidly at 50% moisture. This was attributed to a potentially higher gas exchange rate, but it was noted that higher moisture content may be required to induce and support fruiting bodies. Shen and others (2008) also studied substrate moisture content, and found that biological efficiencies for logs with moisture contents of 50 or 55% had a BE 14-21% greater than those grown at 60% moisture. As in the previously mentioned study, it was concluded that higher moisture levels prevent the block from exchanging air as effectively as blocks with lower moisture levels.

The size of the hard wood chips used may also play an important role in the gas exchange rate of the substrate. Royse and Sanchez-Vazquez (2001) suggest that smaller particles of wood may compact more, leaving less room for gas to exchange freely. This is demonstrated by their study which showed that shiitake substrates with wood chip particles smaller than 0.85 mm were found to have significantly lower biological efficiency (87.1%) than those with larger particles ranging from 0.85 to 4.0 mm (104-107.4%).

Shen and others (2008) discuss the importance of the mushroom growing bag, as well as its roll in proper gas exchanges for the maturing mushroom block. The purpose of the mushroom bag is to keep the inoculated substrate free of

contaminants, and to maintain the desired moisture content through the spawn run. The bags are specially designed to withstand heat, and are typically equipped with a filter patch to allow gas exchange to occur. Some growers prefer to leave the bags on until the block is ready to enter the fruiting chamber (brown in bag), while others remove the bag part way through the spawn run in order to brown the block outside of the bag.

Carbon dioxide levels are known to be important to the growth of shiitake mycelium when browned outside of the bag, however recent studies have demonstrated that it may be important to those browned in the bag as well. Varying sizes of filter patches on the mushroom bags allow for different gas exchange rates, thus affecting the amount of carbon dioxide build up in the bag. Medium and small sized filter patches proved more effective than large patches at preventing contamination and allowing for adequate air exchange. It is suggested that the filter size can also have an effect on the total mushroom production, as well as the likelihood of contamination after the bag is removed (Donoghue and Denison 1995). In their study, Donoghue and Denison (1995) showed that no significant difference was found between the vegetative growth of blocks grown in low carbon dioxide/high oxygen environments versus those grown with aeration the final 5 d of an otherwise high carbon dioxide and low oxygen incubation period. Their main concern was how this would affect the BE and mushroom production of the blocks. It was demonstrated that faster growing strains required a larger patch to provide for higher gas exchange rates than those with a more lengthy vegetative growth period. In order for commercial shiitake production to be optimized, producers should investigate what carbon dioxide and oxygen ratios are preferred by their respective strains. This will allow for the utilization of an

appropriately sized filter patch on the mushroom bag, and prevent excess contamination by molds during the fruiting process.

It should also be noted that the vegetative growth rate is not always directly related to the fruiting body development, and should not be used as a predictor for the overall success of mushroom growth on the block (Royse Sanchez-Vazquez 2001), although it has been suggested that rapid colonization of the substrate may have a positive impact on mushroom yield (Zervakis and others 2001). It is possible that the mycelium on the interior of the block is less developed than the mycelium on the outer surface. As the vegetative growth occurs during the spawn run, it is important that the spawn run is of sufficient length for the mushroom block to fully mature before the fruiting cycle is induced. A spawn run of 116 d resulted in a BE two to three times greater than that of only 58 d. The increased productivity and larger size of mushrooms produced on the more mature logs may be influenced by a number of factors. Some areas to consider are that greater mycelia biomass can better utilize the substrate by releasing more enzymes into the substrate to break down the woody components, greater solubility of wood constituents used in the substrate, or a combination of these factors (Royse 1985). That being said, a short spawn run can also prove to be beneficial by reducing the amount of time that the substrate is at risk for contamination (Zervakis and others 2001).

Food Processing Byproducts and Shiitake Production

According to Royse and Sanchez (2007), the cost of shiitake substrate components has increased due to a high demand for sawdust in other industries, as sawdust is also commonly used for animal bedding, smoking meat, conditioning and mulching soil, production of particleboard and even the production of animal

feed. In hopes of developing a more cost effective production method for growing shiitake mushrooms, some agricultural byproducts have been evaluated to determine whether or not they could act as a suitable replacement for the hardwood component of shiitake substrate. Byproducts from some industries have proven successful as either a partial or full substitute for hard wood in synthetic logs including maize (Philippoussis and others 2003, 2007), olive (Zervakis and others 2001), grape (Gaitán-Hernández and others 2006), eucalyptus (Silva and others 2005), hazelnut (Özçelik and Pekşen 2007), peanut (Zervakis and others 2001), and wheat production (Gaitán-Hernández and others 2006; Philippoussis and others 2007; Royse and Sanchez 2007). Many of these agricultural residues are available virtually free of cost, as they are currently disposed of in landfills or are burned.

The burning of agricultural and forestry residues has been a significant source of environmental contamination. In some countries, such as China, the practice of burning unwanted wastes has led to so much environmental pollution that even airplane traffic is prevented from landing due to the smog and particles in the air. It was estimated that between 20 and 25% of Beijing's three million tons of agricultural and forestry wastes are burned each year. In an effort to minimize the amount of burning in Beijing, unwanted crop straw and tree branches were incorporated into substrate for edible mushrooms, and it was determined that not only was the quality of the mushrooms equivalent or even better than those grown traditionally, but the cost of the substrate was reduced by 15-20% (Zheng and others 2002). These benefits were in addition to the reduction in environmental pollutants, which directly resulted in the reapportioning of the previously unwanted byproducts.

In addition to the environmental and financial benefits of the exploitation of these byproducts, one further advantage is the potential for an increased supply of shiitake mushrooms produced each year. As previously mentioned shiitake mushrooms are an excellent food source and could be used to provide nutrients to people who are otherwise suffering from a lack of food. The increased production will lower prices, making them more affordable to consumers.

In an effort to demonstrate the potential use of the lignocellulosic properties of agricultural and forestry byproducts as raw commodities, or natural resources, rather than undesirable wastes, Chang and Miles (2004) have coined the term "Nongreen Revolution." This phrase represents the potential for using mushrooms (which do not contain chlorophyll and are therefore "nongreen") to utilize this virtually untapped resource, thus minimizing (and eventually eliminating) the environmental hazards associated with their current disposal. They point out that the byproducts of a primary process should no longer be considered as such. Rather, byproducts should be viewed as raw material for secondary and tertiary processes. Mushroom cultivation is an obvious secondary process, but the byproducts of this production could eventually be used as a type of organic fertilizer, thus entirely eliminating the waste stream. Much of China's agricultural land is suffering from the overuse of synthetic fertilizers, and Chang and Miles believe that mushroom byproducts could be used as a phenomenal alternative that will recondition the soil while at the same time providing valuable nutrients for the success of future crops. The potential nutritional, pharmaceutical, and nutriceutical outcome of the "Nongreen Revolution" would supplement the food supply as well as the medical care that is currently available to the rising population.

Pistachio Nuts

Pistachia vera, more commonly known as the pistachio, is a popular commercially grown tree nut with the majority of production found in the United States, Turkey, Iran, and Syria. The green pistachio nuts grow in large clusters, similar to grape bunches, and are harvested in the late summer or early fall. The nuts are encased in a hard shell, which is enveloped by a soft papery skin, known as the hull. As the nuts mature, they naturally split open the hard shell along the suture, and the hull turns reddish pink in color. It is at this point in their development that the nuts are ready for harvest. Immediately after harvest, the hull is stripped from the shell to prevent staining. The majority of the shells are left on, as the pistachios are sold in-shell; however the increasing popularity of pistachio nuts as an ingredient in other foods has created a growing market for shelled nuts. This trend has led to an increase in the number of pistachio shells removed by the processor.

California Pistachio Industry

It is forecasted that the 2009-2010 California pistachio crop will establish the United States as the leader in pistachio production throughout the world with an estimated 175,000 metric tons, followed by Iran with 100,000 metric tons (Figure 1). As pistachios are only grown in a few places in the world, there is a large export market for the nuts. Hong Kong and China are the leading importers of pistachios, bringing in 70,000 metric tons and 27,000 metric tons respectively in 2009-2010 (USDA FAS 2010).

Every other year, pistachios experience what is called an off year. This means that a large harvest will be followed by a smaller harvest the following year. This natural cycle occurs throughout the life of the pistachio tree. Although the 2009-2010 season is expected to be an on cycle year for the California

pistachio industry, California's predicted global domination of the pistachio industry also stems from an increase in bearing acreage as well as ideal environmental conditions throughout the flowering cycle for this crop year. The flowering cycle is a very vulnerable time for the year's pistachio crop. During this time, the fragile buds are vulnerable to heavy rain, hail, drought, and extreme cold conditions. Damage to the flowers can lead to a reduced crop that season, and a combination of these factors can prove devastating to the overall production.

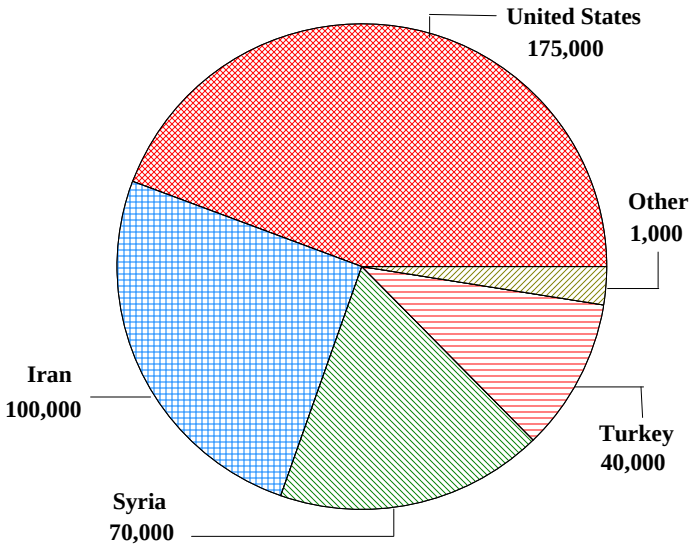

Figure 1 – Worldwide pistachio production in metric tons (Adapted from USDA-FAS 2010)

In addition to the on cycle year and the favorable flowering conditions, the California pistachio industry has significantly increased the number of bearing acres of pistachios, directly resulting in a larger annual harvest. In 1999, there were 71,000 acres of bearing pistachios in California, having risen to 125,637 acres by 2009 (see Figure 2). The total harvest has increased by 232.1 million pounds from 122.4 million pounds in 1999 to 354.5 million pounds in 2009

(Administrative Committee for Pistachios, 2010). With the increase in pistachio nuts harvested each season, the amount of byproducts has risen as well.

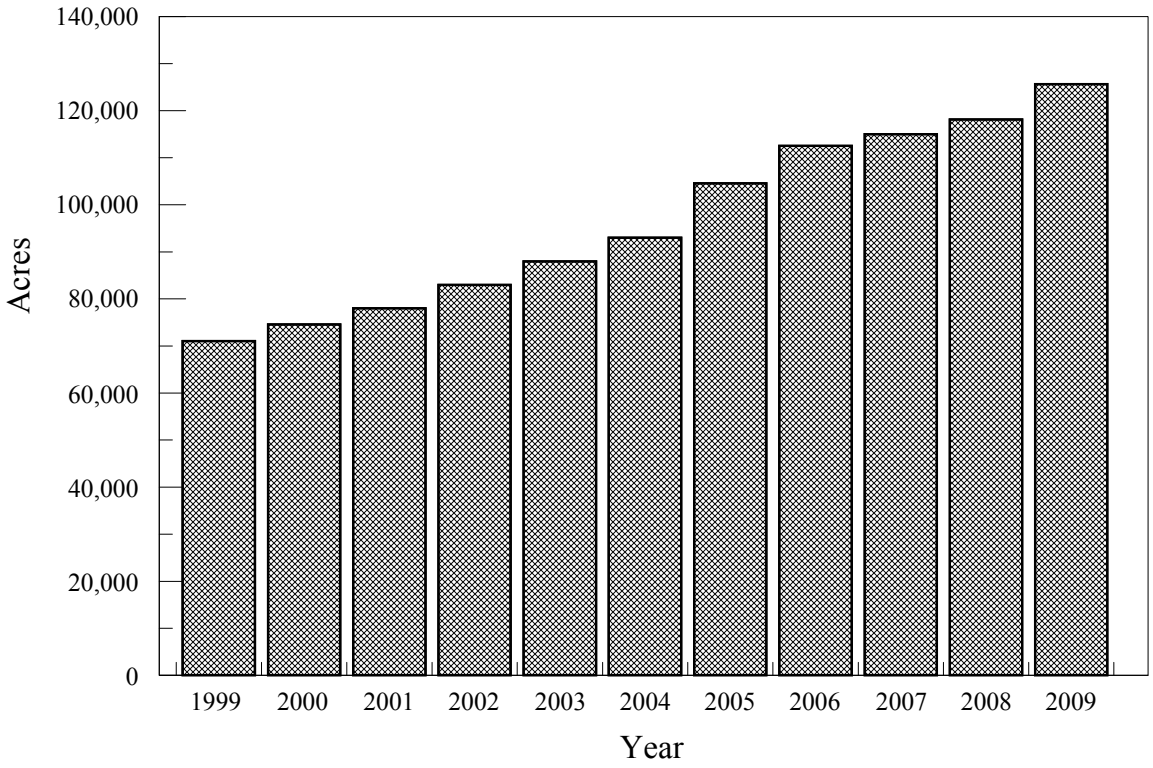

Figure 2 – California pistachio acreage (Adapted from Administrative Committee for Pistachios, 2010)

Pistachio byproducts. Based on our study of the composition of freshly harvested pistachios, it was determined that on a wet weight basis, the total harvest weight is composed of approximately 38.4% hull, 24.9% shell, and 36.5% kernel. Based on these estimations, 63.4 % of the harvest is composed of byproducts (Figure 3).

Figure 3 – Pistachio byproducts (A) shells (B) dried hulls

Until recently, the pistachio industry was fortunate enough to sell their commodity in shell, avoiding the costly disposal of unwanted shells. The hulled nuts were processed and sold in shell, while the grower spread the hulls directly on the orchard. As of late, pistachio nuts have gained popularity as ingredients in value added products, thus increasing the pistachio waste stream as they must be shelled prior to distribution. Two of the most common disposal methods include burning the shells or depositing them in a landfill, causing both environmental and monetary concerns for the producer. Shells have also been disposed of by grinding them into a fine powder and adding it as an indigestible solid matter filler in cattle feed. Finding a use or disposal method for pistachio shells is a prominent concern within the pistachio industry.

Current Uses of Nut Byproducts

Black walnuts are one nut that has developed a fairly significant market for its byproducts. The ground shells have been used since the 1930s to clean airplane engines in a process very similar to sand blasting. They have also proven useful to the auto industry as the metal engine parts can be de-burred, polished, and finished

with ground shells. The use of the walnut shells significantly decreasing the amount of manual labor that would otherwise be required to manufacture automobile parts. Walnut shells are also used to create textured paint, non slip surfaces on walkways, and to clean concrete (Cavender 1973).

Some nut shells have also been tested to determine their suitability as a raw ingredient for activated carbon products. Aygün and others (2003) tested almond, hazelnut, and walnut shells while Ahmedna and others (2004) tested the walnut and almond shells in addition to pecan shells. While it was shown that activated carbon products could be made from the shells, the latter study showed that steam activated shells were a very successful water filter. The steam activated charcoal made from the nut shells was even more successful at removing chlorine byproducts in drinking water than several commercial water filters.

In contrast to black walnut shells, no useful method of utilizing pistachio byproducts has been developed to date. Environmentally friendly disposal methods are also non-existent, thus it is the objective of this study to develop an environmentally friendly use for pistachio byproducts by incorporating them into mushroom substrate.

Chapter 3

MATERIALS AND METHODS

Materials

The materials used in this study are described in Table 3.

Equipment Used in Shiitake Cultivation

Tables 4 and 5 describe the equipment used in this study. Some of the equipment was modified at California State University, Fresno as described below.

Temperature and Humidity Controlled Incubators

Two incubators with the capacity to control temperature, were modified to allow for humidity and light control (Figure 7). The chambers were fitted with 19 L water columns, a system of vinyl piping, and air pumps to allow humidity to be added to the chamber. The water used to fill the water columns was first passed through a UV purifier (Atlantic Ultraviolet Corporation, Hauppauge, N.Y.) to prevent contamination of the growth chamber system. The columns were fitted with heat bands to allow the water temperature to be adjusted, ensuring that proper humidity levels could be maintained within the incubator. Air was pumped into a diffuser at the bottom of the water column, where it bubbled up to the top, collecting moisture as it went. The humidified air then passed through vinyl tubing and into the interior of the incubator (Figure 8). To maintain a high humidity level, and minimize the risk of contamination, a Plexiglass box was constructed to fit onto each shelf. The humid air was piped directly into the boxes,

Table – 3 Summary of materials

Material	Source	Address	Purpose
Solstice Strain Shiitake Mushroom Spawn	Fungi Perfecti	Olympia, Wash.	Colonization of substrate material
Agar Powder	Fisher Scientific	Pittsburgh, Pa.	Component of malt yeast extract agar
Malt Extract Broth	Difco Laboratories	Detroit, Mich.	Component of malt yeast extract agar
Yeast Extract	Fisher Scientific	Pittsburgh, Pa.	Component of malt yeast extract agar
Hulled Barley	SunRidge Farms	Pajaro, Calif.	Barley Spawning
Calcium Carbonate	Sigma-Aldrich	Saint Louis, Mo.	Barley Spawning and Substrate Formulation
Oak Chips	O.C. Inc	Pinketon, Ohio	Control Substrate
Oak Sawdust	Frantz Company	Milwaukee, Wis.	Control Substrate
Wheat Bran	Cargill	Minneapolis, Minn.	Mushroom Substrate
Pistachio Hulls	Nichols Farms	Hanford, Calif.	Experimental Substrate
Pistachio Shells	Horizon Growers	Tulare, Calif.	Experimental Substrate
Mushroom Bags	Fungi Perfecti	Olympia, Wash.	Contain inoculated mushroom substrate
Spring Water	Arrowhead Mountain Spring Water	Wilkes Barre, Pa.	Induction of fruiting cycle
Ice	Produced at CSUF	Fresno, Calif.	Induction of fruiting cycle
Mason Jars	Ball Corporation	Broomfield, Colo.	Containment of barley spawn
Mason Jar Filters	Fungi Perfecti	Olympia, Wash.	Air exchange in Mason jars
Alcohol	Fisher Scientific	Pittsburgh, Pa.	Sterilization of surfaces

Table 4 – Equipment used to create mushroom growing environments

Equipment	Source	Address	Purpose
Desktop Incubator model 146E	Fisher Scientific	Pittsburgh, Pa.	Storage of mushroom spawn on agar and barley mediums
Low Temperature Diurnal Illumination Incubator model 2015	Sheldon Manufacturing Inc	Cornelius, Ore.	Storage of mushroom bags after inoculation
Low Temperature Incubator	Fisher Scientific	Pittsburgh, Pa.	Maintain fruiting conditions
Air Pumps model DAA	Gast Manufacturing Inc	Benton Harbor, Mich.	Pump humid air into incubator
Fluorescent Lights	Good Earth Lighting	Wheeling, Ill.	Provide light during fruiting
UV Purifier model S14A	Atlantic Ultraviolet Corp.	Hauppauge, N.Y.	Sterilize water

Table 5 – Summary of equipment used

Equipment	Source	Address	Purpose
Impulse Sealer	Fungi Perfecti	Olympia, Wash.	Seal mushroom bags
Laminar Flow Hood	The Baker Company Inc	Sanford, Maine	For use when using aseptic techniques
Power Drill	DeWalt Industrial Tool Co	Baltimore, Md.	Mixing of substrate
Universal mixing paddle	Brutus	Boca Raton, Fla.	Mixing of substrate
LCD Digital Hygrometer model no. 3309-50	Cole-Parmer Instrumental Company	Vernon Hills, Ill.	Measured air humidity
Dual-scale Light Meter model no.840020	Sper Scientific	Scottsdale, Ariz.	Measure light
Digital Moisture Analyzer model P26900INST2	CSC Scientific Co	Fairfax, Va.	Moisture evaluation
Vacuum Oven	Fisher Scientific	Pittsburgh, Pa.	Moisture evaluation
Autoclave model STM-E	Market Forge Co	Everett, Mass.	Sterilization of media
Alcohol Lamp	Fisher Scientific	Pittsburgh, Pa.	Sterilization of scalpels
Scalpel	Fisher Scientific	Pittsburgh, Pa.	Cutting agar and transferring mycelium
Digital Balance model PL3002	Mettler Toledo	Columbus, Ohio	Weighing substrates, blocks, and mushrooms
Spectrophotometer model CM-700d	Konica Mindolta	Tokyo, Japan	Mushroom color measurement
Texture Analyser model TA.XT. Plus (Figure 4)	Texture Technologies Corp	Scarsdale, N.Y.	Texture analysis of mushrooms
C:N Analyzer (Figure 5)	TruSpec® CN	St. Joseph, Mich.	Carbon and nitrogen estimation
Goldfisch Fat Extraction Apparatus model 3500100 (Figure 6)	Labcono Corporation	Kansas City, Mo.	Fat determination in substrate components
Muffle Furnace model 51828	Lindberg	Riverside, Mich.	Ash Analysis

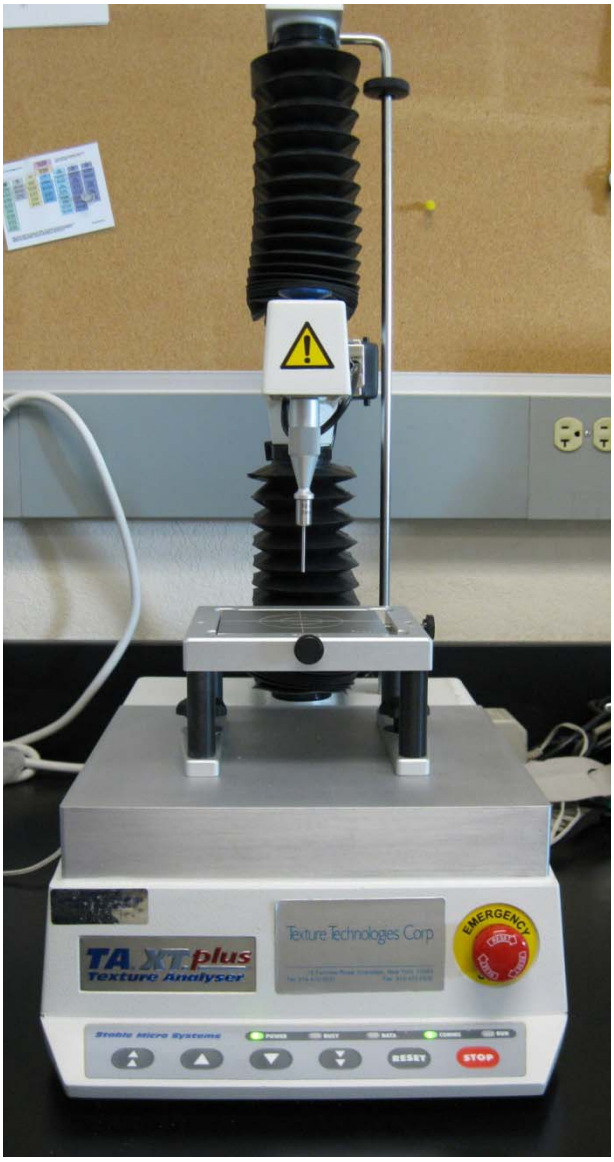

Figure 4 – Texture Technologies Corp TA. XT. Plus Texture Analyser fitted with 2 mm probe

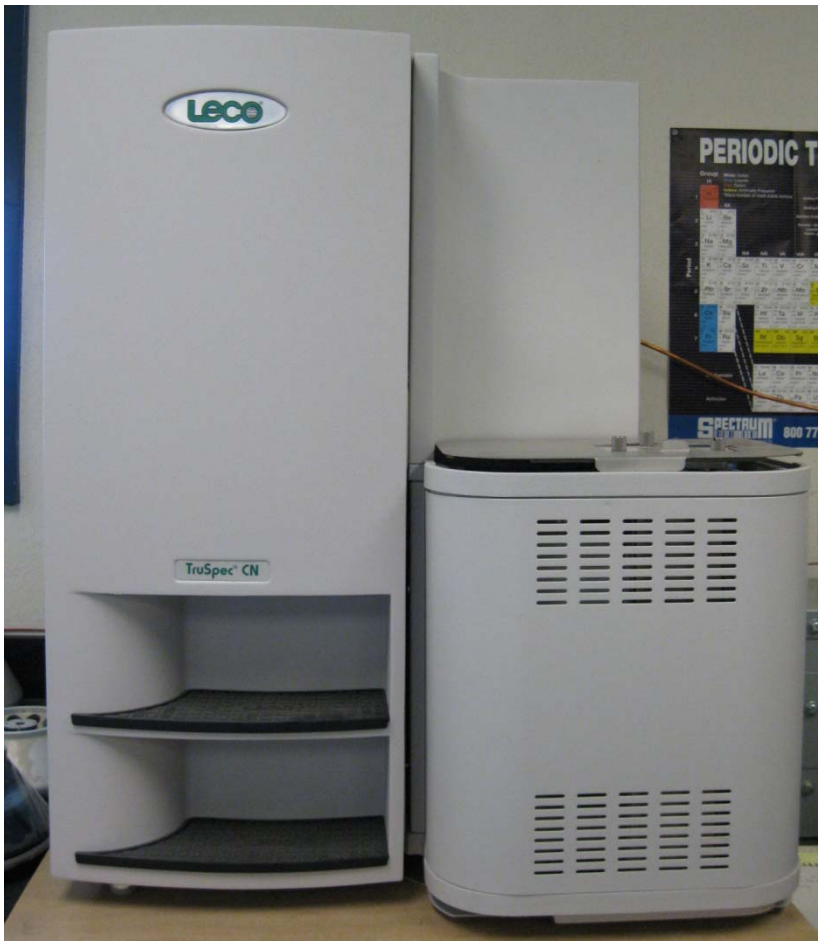

Figure 5 – Leco TruSpec CN Analyzer

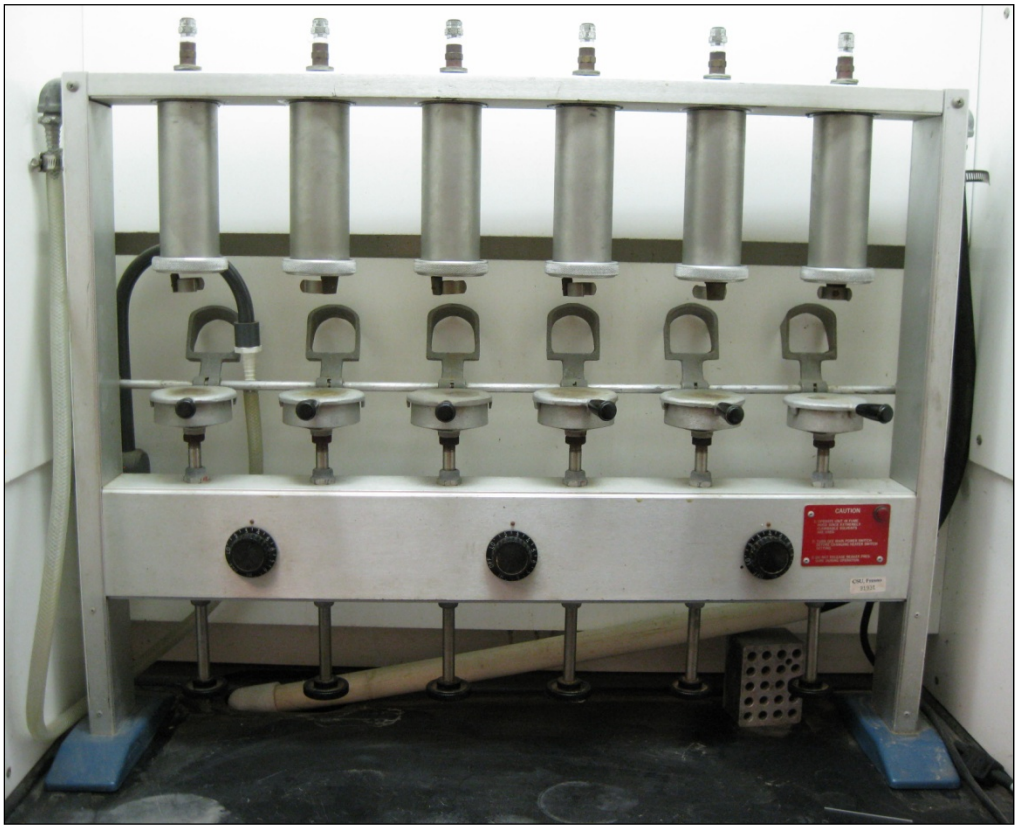

Figure 6 – Goldfisch fat extraction apparatus

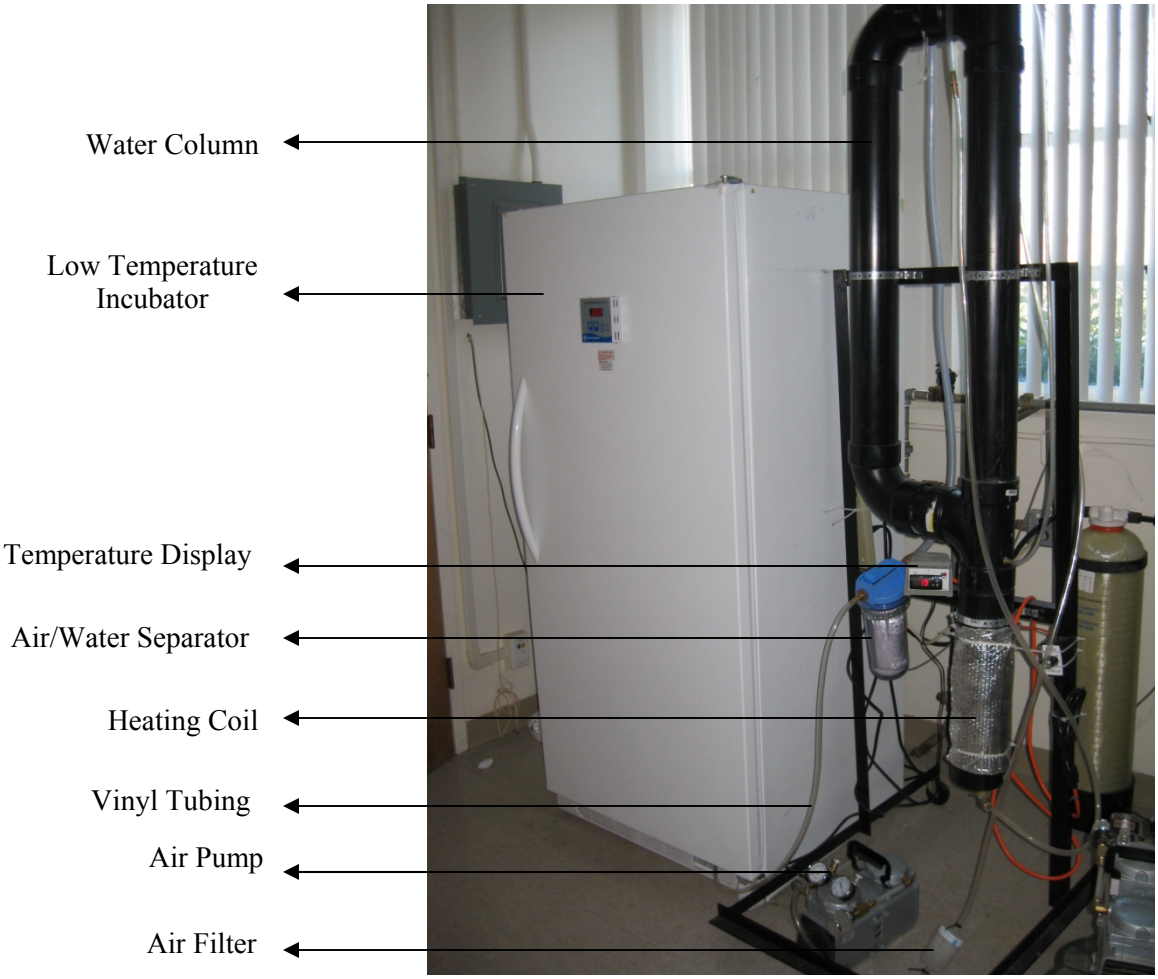

Figure 7 – Low temperature incubator equipped with water column and air pump

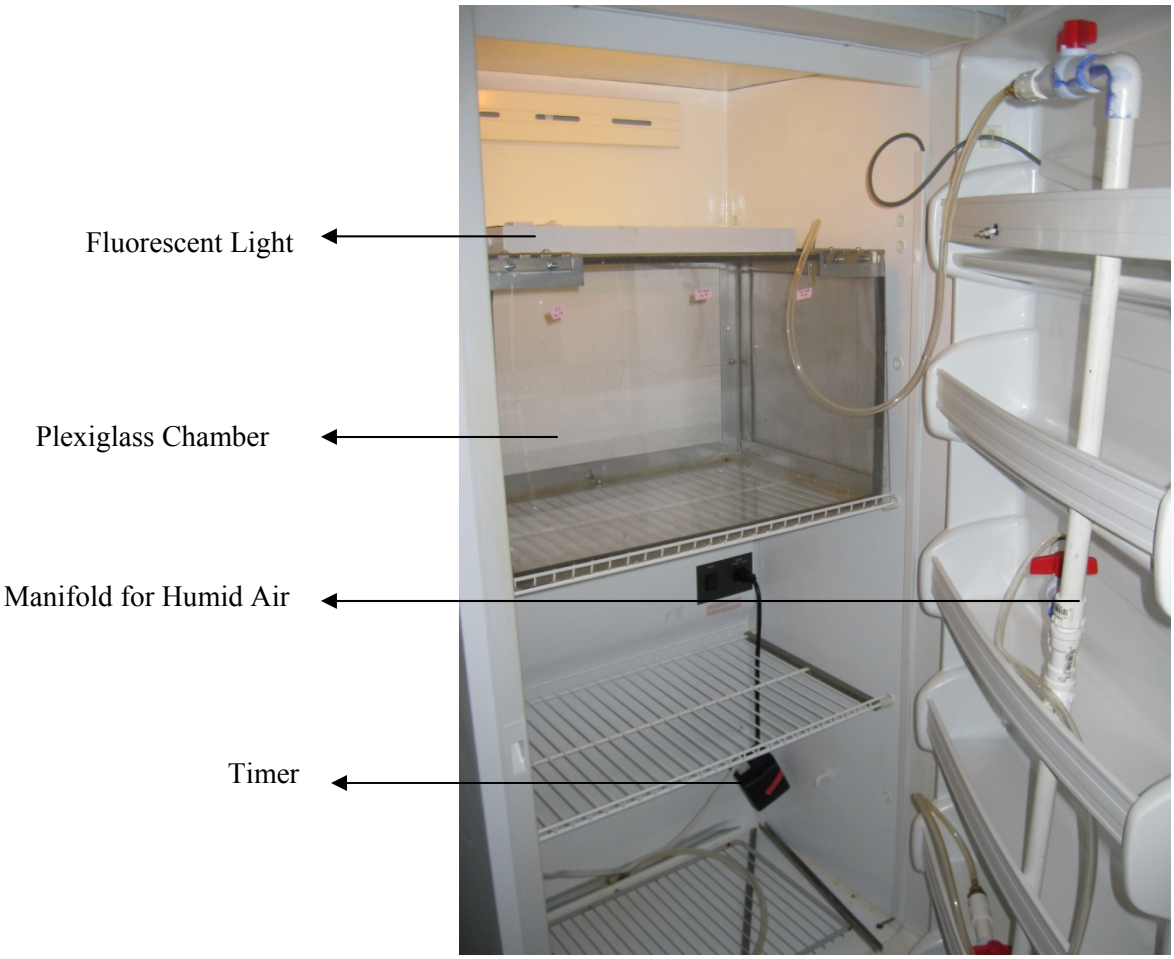

Figure 8 – Mushroom growth chamber environment

creating a positive flow of humid air out of the box. Light was provided by a fluorescent tube (0.5 m, 15 W) that rested on top of the Plexiglass box.

Methodology

Collection of Substrate Materials

The pistachio hulls (Nichols Farms, Hanford, Calif.) were collected and dried in the sun for one week to reduce the moisture content and allow for easy storage.

Preparation of Agar Media

Malt Yeast Extract (MYE) agar was utilized to maintain the Shiitake spawn culture. This was made by mixing 15 g malt extra broth, 15 g bacto agar, and 5 g of yeast extract in 1 L deionized water and autoclaving for 20 min to sterilize the media.

Preparation of Mushroom Spawn

Pure shiitake mushroom culture was obtained from Fungi Perfecti (Olympia, Wash.) and stored at 24°C in a table top temperature controlled incubator (Fisher Scientific, Pittsburg, Pa.). The Solstice Strain was selected for its ability to grow at temperatures up to 24°C, which is important considering the environmental conditions of California's Central Valley. The shiitake culture was maintained on MYE agar by transferring a 1 cm x 1 cm square from a colonized plate to the center of a new plate using sterile technique under the laminar flow hood (The Baker Company Inc, Sanford, Maine) every 2 wk. This process ensured that the mushroom culture remained vigorous and had a good supply of nutrients available at all times. After a 2-wk incubation period, the mycelium spread across the surface of the agar, and was ready to be transferred onto barley

substrate. In preparation for the transfer, the agar was cut into 1 cm x 1 cm squares using sterile techniques under the laminar flow hood.

Barley spawn. During the course of the experiment, two different sizes of mushroom substrate blocks were used to cultivate the mushrooms. Therefore, the amount of barley spawn needed to inoculate the substrate varied. For medium bags, 235 g of hulled barley (SunRidge Farms, Pajaro, Calif.), 0.5 g calcium carbonate (Sigma-Adrich, Saint Louis, Mo.) and 200 ml of deionized water were mixed in a 32 oz wide mouth Mason jar (Ball Corporation, Broomfield, Colo.). For small bags, 117.5 g of hulled barley, 0.25 g calcium carbonate, and 100 ml of deionized water were mixed in a 32 oz wide mouth Mason jar. The jars were capped with ring lids and filters (Fungi Perfecti, Olympia Wash.), to allow adequate gas exchange during the barley spawning process. The Mason jars were sterilized for 60 min at 121°C (103 kPa) twice, with 24 h between heat treatments.

Inoculation of barley. Once the jars were cool to the touch, they were vigorously shaken until the barley was broken up into individual grains. The aforementioned agar squares were then scraped into the jars under the laminar flow hood, and the filter and ring lid were reattached (Figure 9). The agar squares were distributed throughout the barley by gentle shaking, and the jars were stored at 24°C for 2 wk. To prevent the mycelium from forming a large clump at the bottom of the jar, the jars were vigorously shaken at the end of each week. At the close of wk 2, the barley spawn was developed enough to inoculate the mushroom substrate.

Figure 9 – (A) Inoculated barley jar (B) agar has been gently distributed throughout.

Analysis of Substrate Material

Moisture absorption test. Pistachio shells were soaked in water. At 1 h intervals, a small sample was removed, blotted with a paper towel to remove excess surface water, and placed in the digital infrared moisture analyzer to determine the moisture content. This procedure determined the water holding capacity of the pistachio shells, which was very useful when adjusting the substrate to the proper moisture level.

Carbon nitrogen analysis. Substrate components were ground and dried in a vacuum oven for 24 h. Three samples of approximately 175 mg in weight were measured out and prepared for analysis from each substrate component. The CN analyzer was calibrated using both an alfalfa standard (Carbon: 45.17% ± 0.31, Nitrogen: 3.32% ± 0.04) and an oat standard (Carbon: 46.85% ± 0.40, Nitrogen:

2.70% ± 0.04). The standards were also run with approximately 175 mg per sample.

Ash analysis. The ash content of pistachio shells and hulls were determined using the methods approved by AOAC International (2000). Approximately 5 g was ignited for 2 h at 550ºC, and the ash content was determined as follows:

$$\% \text{ ash (dry wt basis)} = \frac{\text{Wt after ashing-Tare wt crucible}}{\text{Original sample wt} \times \text{dry matter coefficient}} \times 100$$

where dry matter coefficient = % of solid÷100

Carbohydrate. Carbohydrate was determined by method of difference (AOAC 2000).

Preparation of Substrate Materials

Pistachio shell preparation. The pistachio shells were soaked in tap water for 24 h in preparation for their use in mushroom substrate. They were then strained into a mesh colander and allowed to drip for 10 min before being added to the other substrate components.

Substrate. Six different mushroom substrates were developed: one control and five experimental. All six substrates had the same general set up: 40% wheat bran, 60% woody substrate, and enough water to bring the moisture level to between 50-55%. In addition to the wheat bran, the blocks were also supplemented with 0.6% calcium carbonate (on a dry weight basis). The components of each substrate were added to a 5 gal bucket and thoroughly mixed with a drill motor (DeWalt Industrial Tool Co, Baltimore, Md.) equipped with a universal paddle

mixer (Brutus, Boca Raton, Fla.). The mixing process took 5 min, ensuring that the moisture and substrate components were as thoroughly blended as possible. A small sample of the substrate was collected for analysis of moisture content in a vacuum oven (Fisher Scientific, Pittsburgh, Pa.). The substrate was then scooped into a mushroom bag (Fungi Perfecti, Olympia, Wash.), at which point the top of the bag was folded over several times and tied off with string before being placed in the autoclave (Market Forge Co, Everett, Mass.). The substrate bags were sterilized for 60 min at 121°C (103 kPa) twice, with 24 h between each treatment. Two control substrate bags were made at the start of each trial, as the blocks were divided between two growth chambers during the fruiting cycle. This allowed for a control to be present in each chamber. Figure 10 describes the cultivation process.

Inoculation of substrate. Once the substrate bags reached room temperature under the laminar flow hood, they were inoculated with the colonized barley. The barley jars were wiped down with alcohol to prevent contamination of the bags. The string was removed from the mushroom bag and the bag was carefully unfolded, to minimize the risk of tearing the filter patch. The colonized barley was poured into the mushroom substrate, and the bag was immediately sealed using an impulse sealer (Fungi Perfecti, Olympia, Wash.). Multiple seals were made as close to the top of the bag as possible. This ensured that there was an adequate amount of headspace inside the bag to aid in proper gas exchange.

The mushroom substrate, having formed a fairly compressed block in the autoclave, was gently broken up and mixed with the barley spawn. The substrate and spawn were mixed by manual manipulation and gentle shaking from the outside of the bag, until the barley could be seen all sides of the substrate and the

substrate was no longer compressed into a block. The sealed bags were then placed in a 24ºC incubator (Sheldon Manufacturing Inc, Cornelius, Ore.).

Spawn run. The inoculated mushroom bags were left in a dark 24ºC incubator undisturbed for about 30 d, to allow the mycelium fibers to knit around the substrate. By the end of the 30 d, the blocks that possessed a thick, bumpy, white layer of mycelium (Figure 11), were exposed to a fluctuating night and day environment. The day time conditions consisted of 16 h of 24ºC temperature, and 8 h of light provided by four full spectrum bulbs. The night time conditions consisted of 8 h of 18ºC temperature with no light exposure. This temperature and light cycle was used to simulate the natural environmental changes that a block might experience if grown outdoors, thus inducing primordial formation.

Shock. Once the mushroom blocks began to brown slightly and develop pinning, the bags were removed from the incubator and shocked, to induce mushroom formation. Prior to shocking the blocks, a small slit was cut near the top of the bag and any excess water that had leached from the substrate was drained and weighed. To simulate a soaking shock with as little risk of contamination as possible, the blocks were soaked within their bags. The bag was sliced open at the top and Arrowhead Mountain Spring Water (Wilkes Barre, Pa.) was poured into the bag until the block was completely submerged. The bag was resealed and submerged in ice water for 3 h.

Growth chamber. After the blocks were shocked, they were removed from their bags and placed within the humid Plexiglass boxes inside the growth chamber incubators (Fisher Scientific, Pittsburgh, Pa.) (Figure 8, p. 27). The empty bags were weighed to determine how much mushroom mycelium and

substrate had remained attached to the bag. The temperature and humidity of the growth chamber were maintained at 20 °C and 90-95 % relative humidity respectively, with 8 h of light provided each day by 15 W fluorescent bulbs.

Mushroom fruiting and harvest. Within a week after the shock treatment, mushroom development began. Mushrooms were harvested at the same time each day when the gills are fully exposed and the edge of the caps are still curled under (Figure 12). Mushrooms were gripped at the base of the stem and gently twisted off the block. Any residual mushroom stem was removed from the block, as it may become vulnerable to mold growth.

Mushroom analysis. Immediately after harvest, the mushrooms were weighed and the diameter of the caps is measured. An average minimum and maximum diameter for each block is determined for each harvest day. Several mushrooms from each block were then placed in a vacuum oven for 24 h to determine their moisture content. A texture analysis was conducted on the mushroom caps to determine their firmness. This was conducted by a texture analyzer set to measure the peak force while penetrating 95% of the depth of the sample with a probe that was 2 mm in diameter. A total of thirty texture tests were run on each of the mushroom samples, with five tests on each of six caps from each substrate formulation. The caps were prepared for testing by trimming the stem as close to the gills as possible. The puncture test was carried out on five different areas of the cap, all the while avoiding the stem. Color was also measured on the mushroom caps using a portable spectrophotometer (Konica Minolta, Tokyo, Japan). The spectrophotometer was calibrated using a white plate, and measured the color values using the L*, a*, b* scale where L* is an indication of brightness, a* is range from green to red, and b* is a range from blue

to yellow. In order to evaluate the varying color across the cap, five readings were taken starting at the center and moving along the radius to the edge of the cap. These readings were then averaged to determine the average L*, a*, b* values for the mushroom.

Determining efficiency of the mushroom substrate. After all of the mushrooms have been harvested and the block is no longer producing, the BE of the block must be determined. This is done by dividing the fresh weight of the mushrooms by the weight of the block on a solid basis and converting the resulting number to a percent.

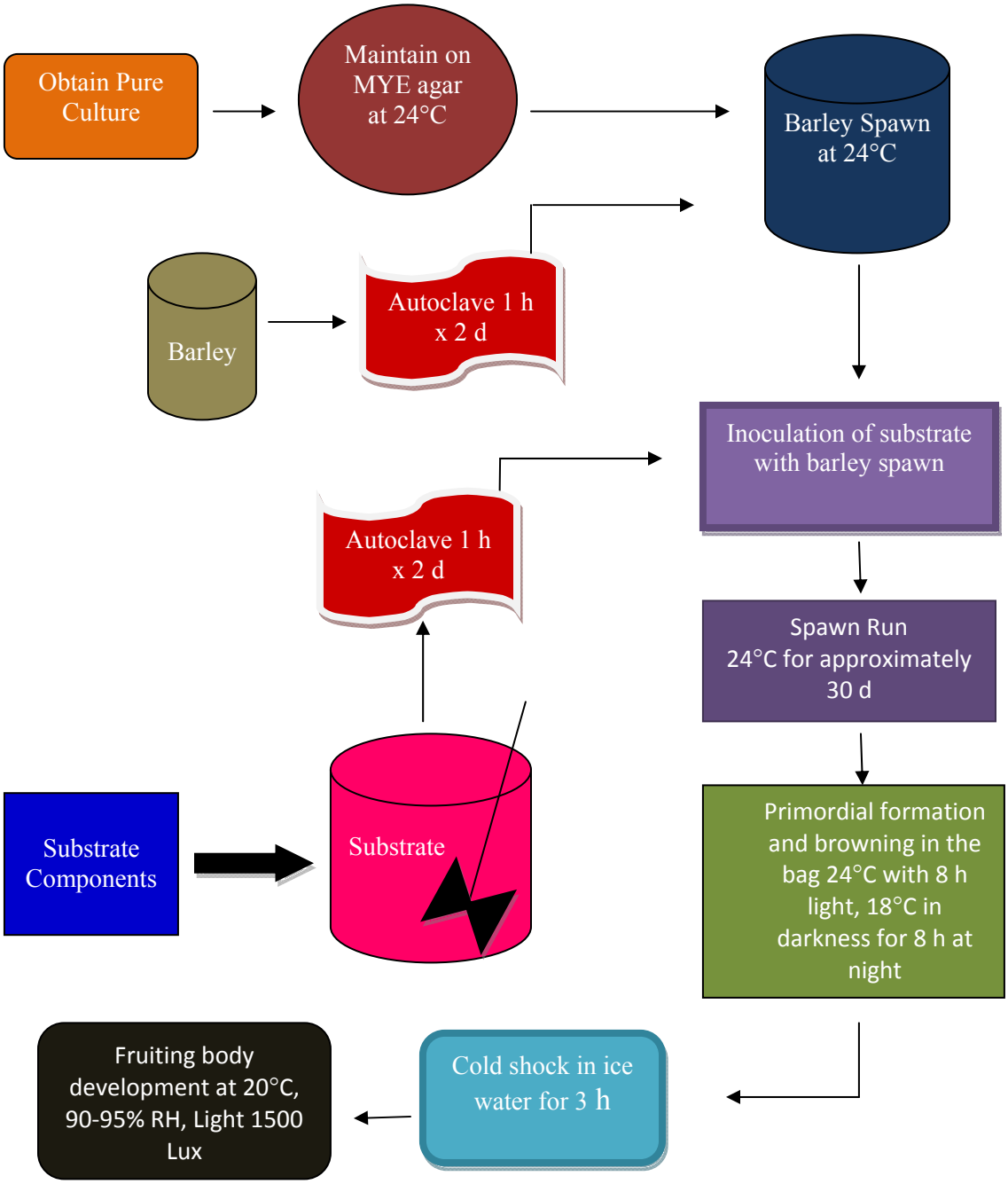

Figure 10 – General flow chart for the propagation and cultivation of shiitake mushrooms

Figure 11 – 50% shell / 50% hull block showing development required before exposure to fluctuating night and day environmental conditions

Figure 12 – Shiitake mushrooms ready for harvest from a control block

Chapter 4

RESULTS AND DISCUSSION

Characteristics of Pistachio Shell, Hull, and Other Components

Proximate Composition of Pistachio Shells and Hulls

The proximate composition of pistachio shells and hulls was not found in the published literature. The appropriate analyses were conducted to determine the protein, carbohydrate, fat, ash, and moisture content of the pistachio shells and hulls (Table 6).

Table 6 – Proximate composition (Mean ± SD) of pistachio shells and hulls used in shiitake mushroom substrate

	Composition (% Dry Weight Basis)				
Material	Moisture	Carbohydrate	Ash	Protein	Fat
Shell	6.0 ± 0.10[a]	98.29 ± 0.03	0.52 ± 0.03	0.56 ± 0.05	0.63 ± 0.08
Hull	7.5 ± 0.06[a]	67.63 ± 0.55	13.27 ± 0.27	14.30 ± 0.11	4.80 ± 0.22

[a]Total weight basis

Water Holding Capacity of Shells

The water holding capacity study was used to establish the appropriate length of time the shells need to be soaked in order to obtain the maximum moisture content. It was found that after 24 h, the moisture content of the shells increased from about 6.9% to 33% (Figure 13).

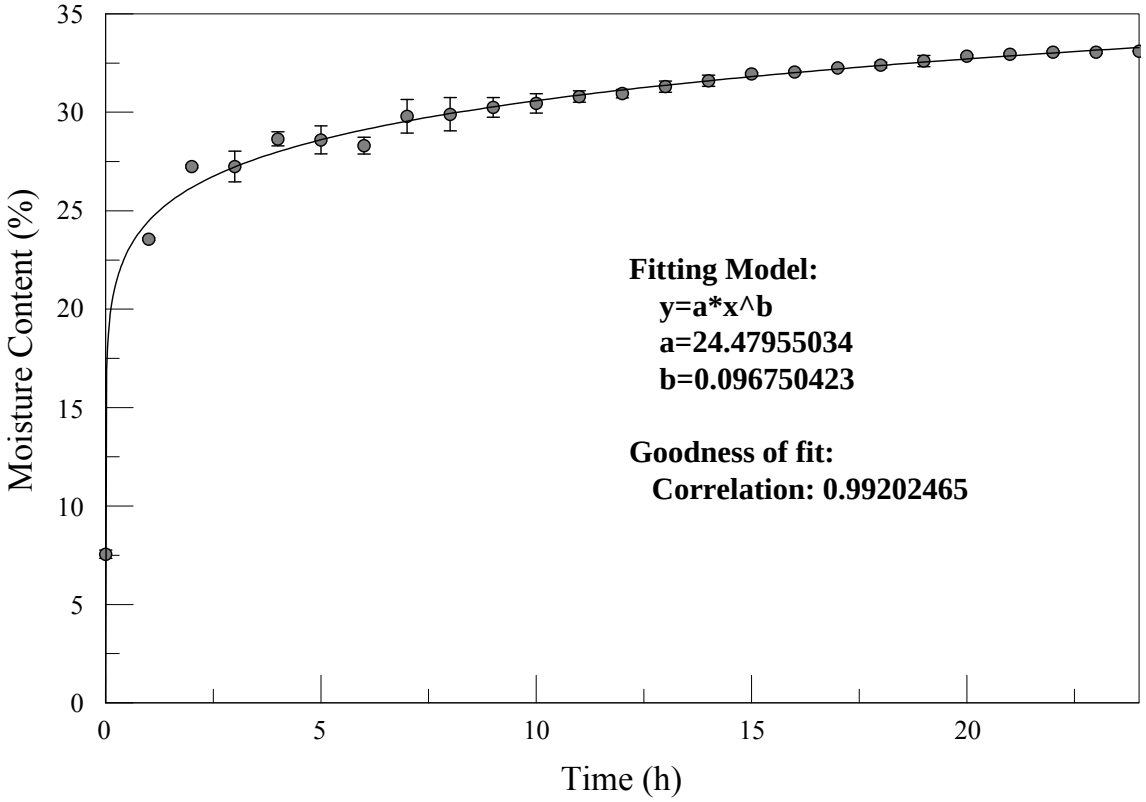

Figure 13 – Water holding capacity of pistachio shells

Moisture Content of Other Components

The moisture content of all substrate components was evaluated. Based on the findings, the moisture content of the dry formulations was adjusted with water to achieve the desired moisture content of 50-55% (Table 7).

Table 7 – Moisture content of non-pistachio substrate components (Mean ± SD)

	Wheat Bran	Wood Chips	Wood Shavings
% Moisture	10.0 ± 0.12%	6.5 ± 0.06%	7.3 ± 0.06%
Coefficent of Variation	1.15%	0.88%	0.79%

Pistachio Shell and Hull as a Substrate for Shiitake Mushrooms

Table 8 provides the formulation for the control and five experimental substrates. No significant difference could be distinguished between the control and experimental substrates throughout the vegetative growth cycle. The mycelium grew throughout the substrate at approximately the same rate, and each block was ready to reach the fruiting cycle at very similar times. Figure 14 demonstrates the similar development of both the control and experimental blocks. Some of the blocks even produced mushrooms more prolifically than the control (Figure 15).

Efficiency of Pistachio Shell and Hull for Growing Shiitake Mushrooms

There are many factors influencing the success of the mushroom block, and it is noted that the BE of some substrates were not consistent over the course of the three trials (Table 9). As mentioned by Royse and Sanchez-Vazquez (2001), the success of the vegetative growth is never an indicator of the potential success of the fruiting cycle. This may explain why some of the blocks appeared to have colonized sufficiently, yet produced few, if any, viable fruiting bodies. This could be a result of the timing of one of our critical cultivation steps, or it could even be related to another important indicator of the mycelium's growth that we are unaware of. The cultivation of Shiitake mushrooms has long been viewed as an art form by the people of its native lands, and it is only recently that the technique has been approached in a scientific fashion. What was once accomplished by intuition, feel, and experience, will require some learning by those who wish to achieve similar consistent results, regardless of the substrate in use.

Table 8 – Substrate formulation for control and experimental blocks

Substrate	Oak Chips	Oak Sawdust	Wheat Bran	Hulls	Shells	Calcium Carbonate	Water
Control	100	200	200	0	0	2.75	520
100% Shell	0	0	200	0	300	2.29	346
75% Shell/ 25% Hull	0	0	200	75	225	2.40	389
50% Shell/ 50% Hull	0	0	200	150	150	2.52	431
75% Hull/ 25% Shell	0	0	200	225	75	2.63	474
100% Hull	0	0	200	300	0	2.75	517

* All values given in grams (g) except water (mL)

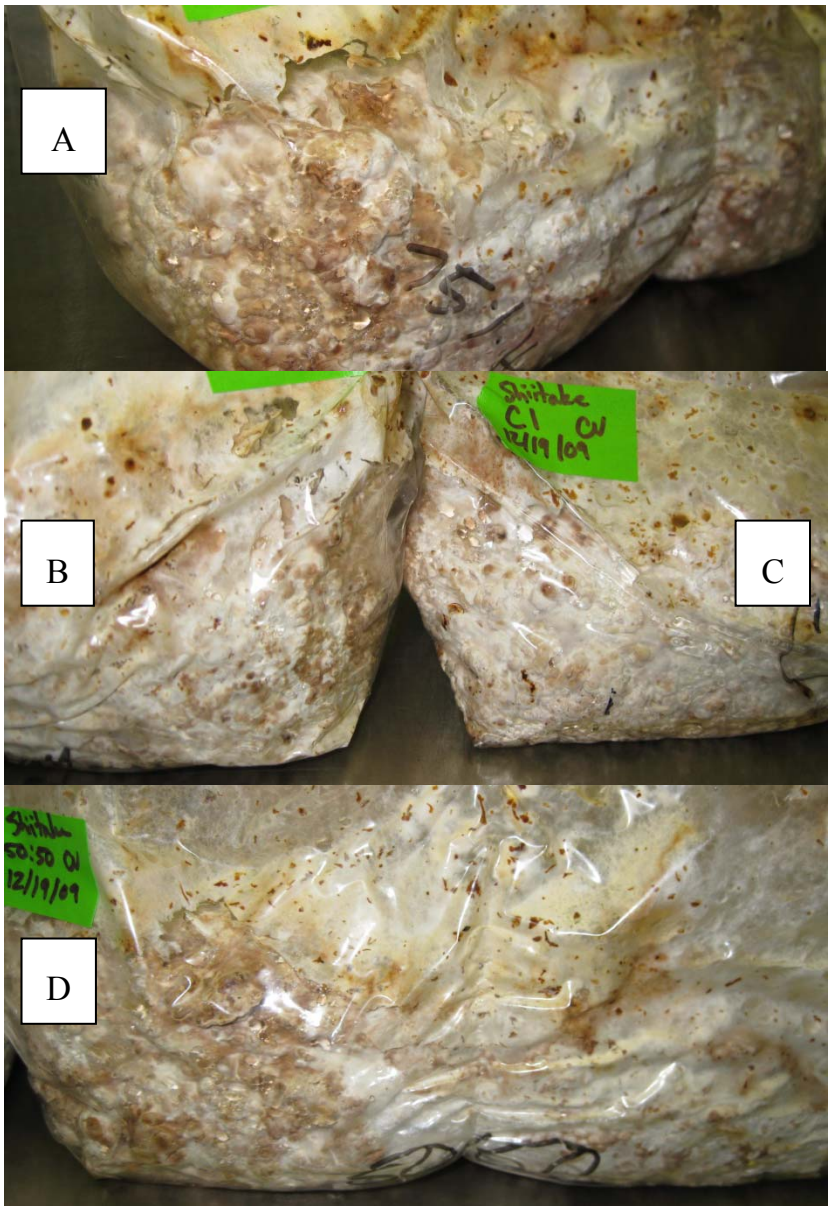

Figure 14 – Mushroom blocks 22 d after inoculation showing similar development: (A) 75% hull / 25% shell, (B) 100% hull, (C) C1, (D) 50% shell/50% hull

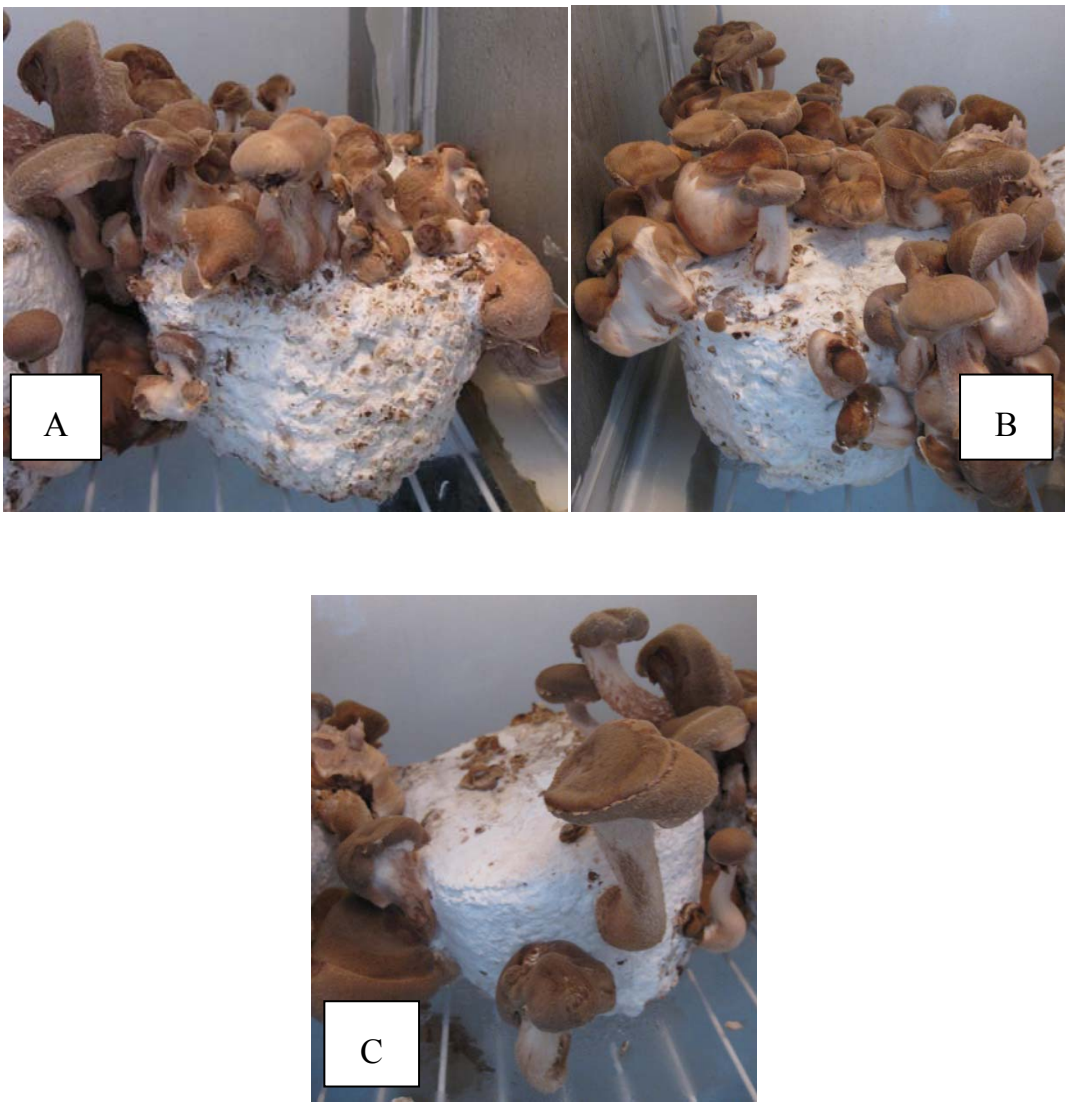

Figure 15 – Experimental blocks A (75% shell / 25% hull) and B (50% shell 50% hull) outperforming C (control 1) in trial 2

Table 9 – Biological efficiency of control and experimental substrates

Trial #	Control 1	Control 2	100% Hull	75% Hull / 25% Shell	50% Shell/ 50% Hull	75% Shell/ 25% Hull	100% Shell
1	20.90	38.80	0.00	51.10	0.00	47.00	0.00
2	49.00	1.60	7.80	12.40	65.10	64.20	1.60
3	24.80	33.20	0.00	40.70	13.60	51.00	21.50
Average	31.57	24.53	2.60	34.73	26.23	54.07	7.70
Standard Deviation	15.22	20.057	4.503	20.028	34.340	9.001	11.978
Range	20.90 - 49.00	1.60 - 38.80	0.00 - 7.80	12.40 - 51.10	0.00 - 65.10	47.00 - 64.20	0.00 - 21.50

Quality of Shiitake Mushrooms Grown on Pistachio Shells and Hulls

Moisture

Variation between the average texture measurements of the mushrooms from the five substrates listed may be influenced by the moisture content of each substrate's respective mushrooms. The moisture content of the mushrooms ranged from 84.62% to 90.58%, which could have the potential to influence the force required to penetrate the caps. Figure 16 demonstrates the moisture content of mushrooms grown on each of the seven substrates. The greater the percentage of shells in the substrate, the higher the mushroom moisture content appears to be, although the 100% shell substrate did not necessarily adhere to this trend, as the moisture content is only slightly higher than that of the 50% shell / 50% hull substrate.

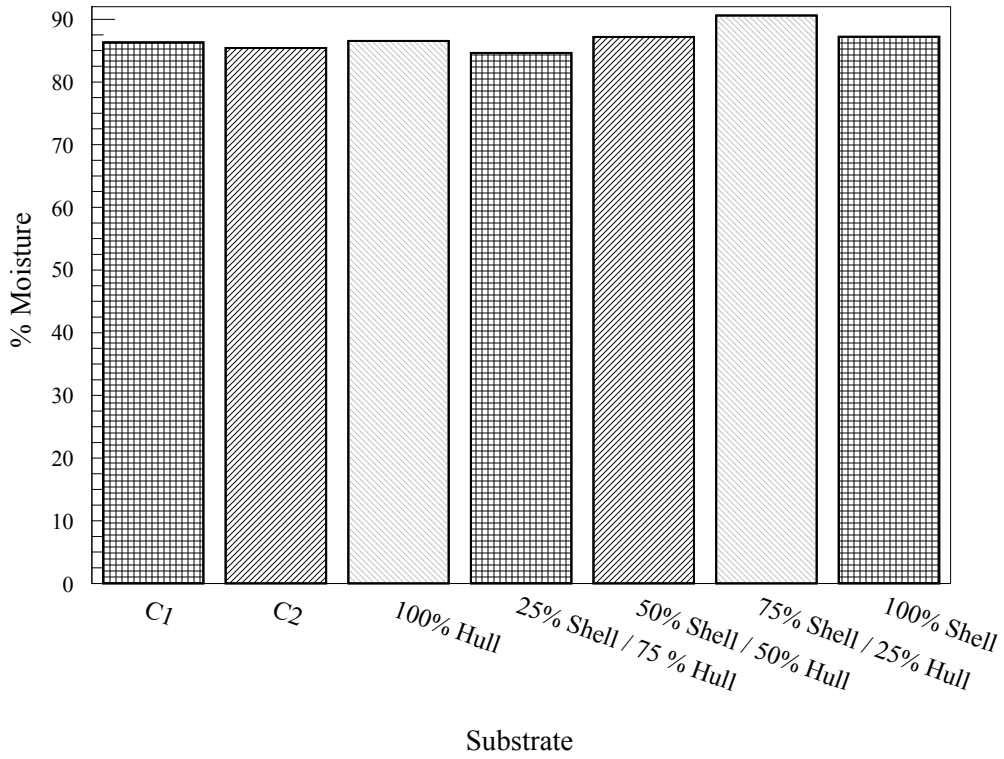

Figure 16 – Moisture content of shiitake mushrooms

Color

The colors present on the mushroom cap are not arranged in a particular pattern. Some caps may appear speckled, striped, or even blotchy in color, with dark and light variations throughout. As a result of this variable color, it is necessary to determine an average color value for the cap (Figure 17) for the mushrooms in the sample (Figure 18). As the 75% shell and 75% hull substrates were the most successful substrates overall, a more detailed description of the overall color for four mushrooms from each substrate is shown in Table 10.

Texture

The force necessary to penetrate 95% of the depth of the mushroom cap was measured for mushrooms grown on two control and three experimental

substrates (Figure 19). The remaining two substrates did not yield mushrooms suitable for this testing, as the mushrooms had very large stems and not enough exposed gill area on which the test could be conducted (Figure 20). Variations between the measured peak forces required to penetrate the cap are thought to result from inherent differences between mushroom caps, as well as the moisture content of the mushroom caps.

Size

The diameters of the mushrooms from each trial were measured to determine the size of the mushrooms produced on each substrate (Table 11).

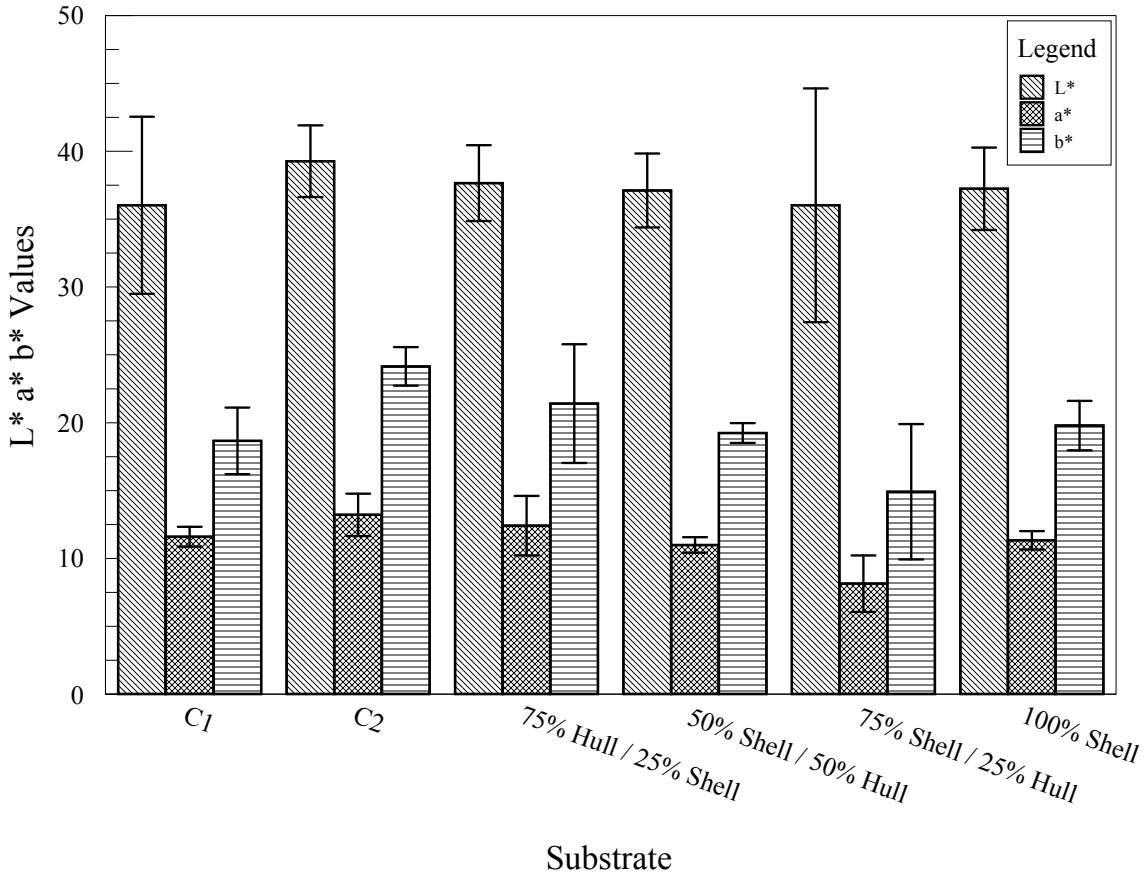

Figure 17 – Average L*, a*, b* values of Shiitake mushrooms grown on experimental and control substrates. Letters within the bars show significance at a 95% confidence interval

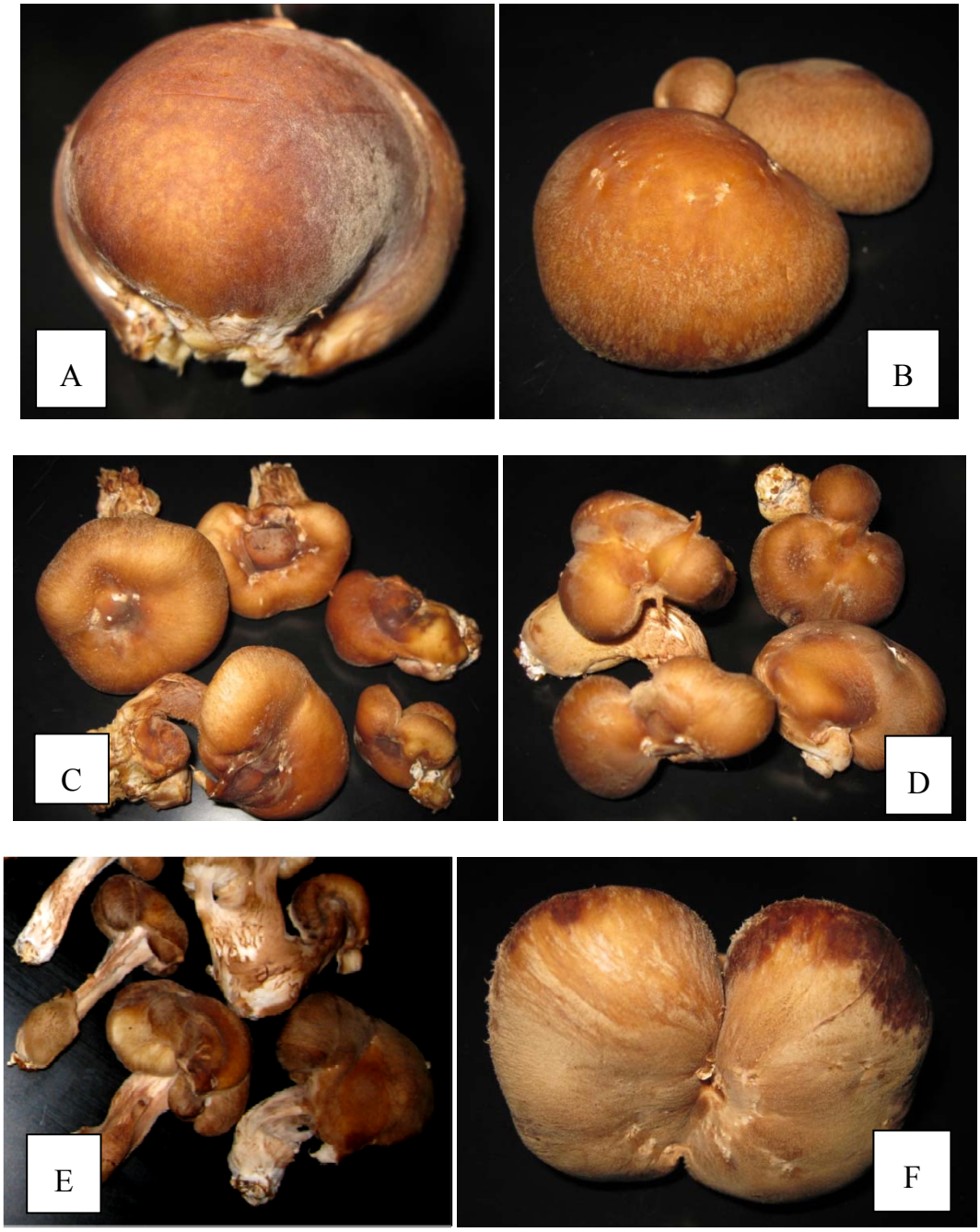

Figure 18 – Trial 5 Shiitake mushrooms displaying varying colors across cap area (A) C1 (B) C2 (C) 75% shell / 25% hull (D) 75% hull / 25% shell (E) 50% shell / 50% hull (F) 100% shell

Table 10 – Range and mean L*, a*, b* color values for 75% hull / 25% shell and 75% shell / 25% hull mushrooms

Substrate		L*	a*	b*
75% Hull/ 25% Shell	Range	36.12 - 51.88	7.75 - 16.26	15.58 - 33.41
	Mean	42.39	12.59	23.35
	Standard Deviation	4.69	2.08	23.35
75% Shell/ 25 % Hull	Range	28.88 - 59.69	8.38 - 16.43	13.00 - 30.44
	Mean	43.58	13.05	24.12
	Standard Deviation	8.14	2.11	5.2

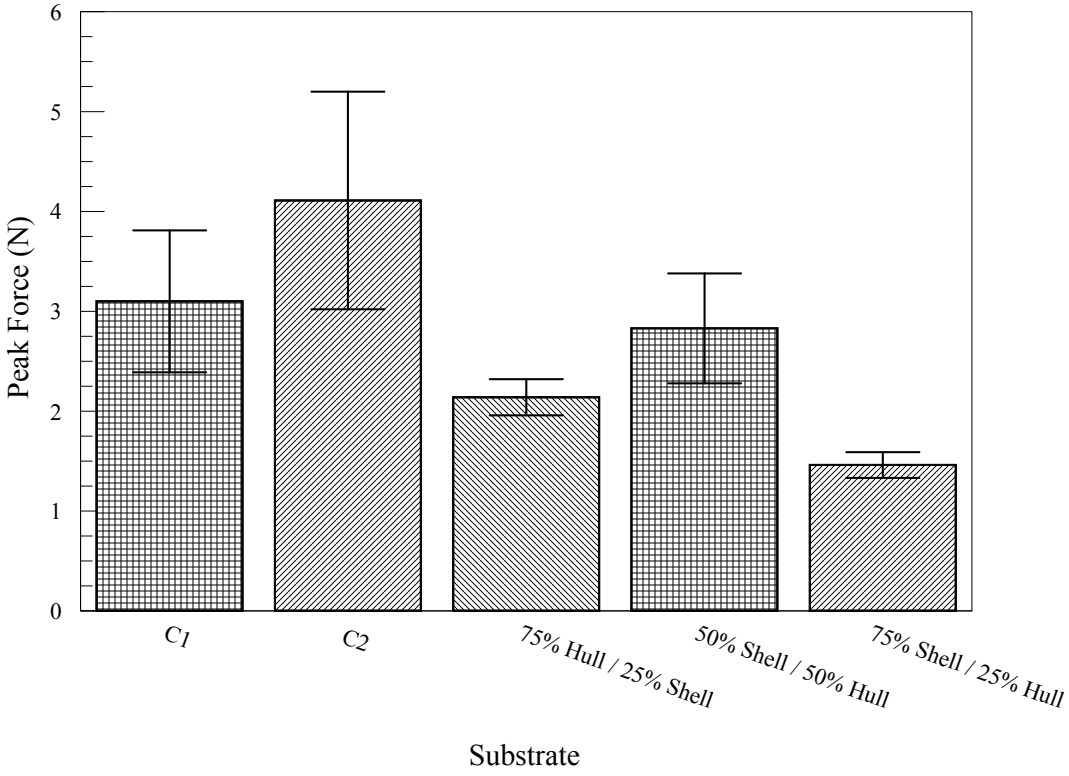

Figure 19 – Peak force required to penetrate 95% depth of shiitake mushroom cap

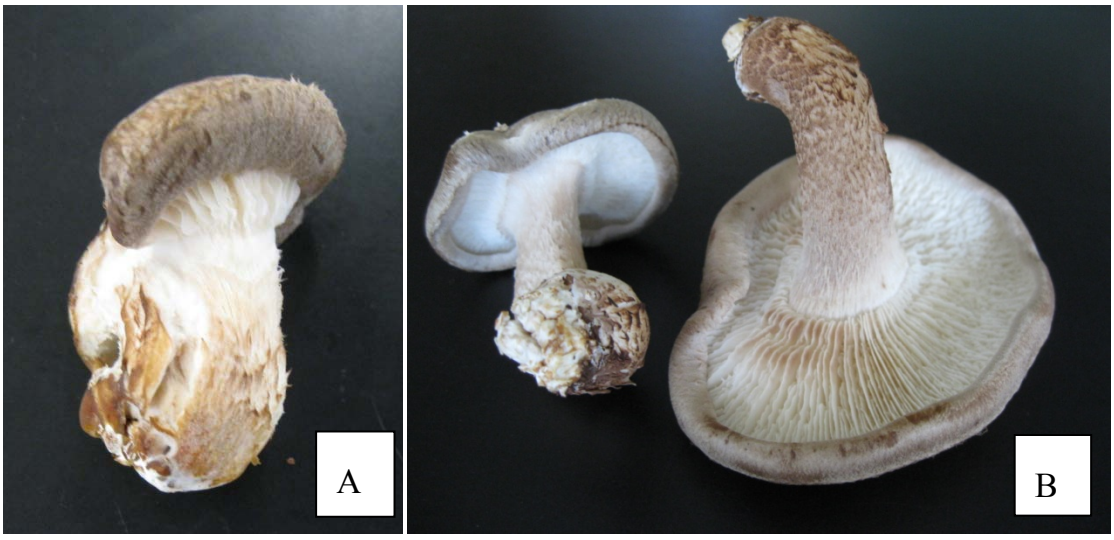

Figure 20 – (A) Mushroom showing enlarged stem and inadequate gill area for texture analysis versus (B) mushrooms showing normal stem and ideal gill exposure

Table 11 – Range of shiitake mushroom diameters for each substrate

Substrate	Range (cm)
Control 1	2.0 - 7.2
Control 2	1.8 - 11.1
100% Hull	0.8 - 4.1
75% Hull	2.0 - 7.7
50% Hull 50% Shell	1.7 - 6.4
75% Shell	1.2 - 6.7
100% Shell	1.8 - 6.6

Chapter 5

CONCLUSION

Pistachio shells and hulls have proven to be a good substrate material for the growth of Shiitake mushrooms. Based on the proximate composition of the shells and hulls, they seem to complement each other quite suitably and offer a variety of nutrients to the Shiitake mycelium. This is especially true when the shells and hulls are used in combination with one another. It was found that the 75% shell/25% hull and 75% hull/25% shell ratios were quite successful, resulting in higher biological efficiencies than the control substrate the majority of the time.

The quality of all mushrooms grown was very similar, regardless of the type of substrate used. Further study needs to be conducted on the specific growing conditions required by the Shiitake mycelium on the pistachio based substrate in order to achieve a more consistent BE.

The successful cultivation of shiitake mushrooms on pistachio shells and hulls proves that mushroom substrate is a viable avenue for pistachio farmers to market the byproducts of their harvest. As nearly two thirds of each harvest is considered inedible and has little or no value, there is significant potential for this research to develop additional revenue for the pistachio industry and to minimize the environmental hazards caused by their disposal.

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