Antimicrobial Testing and Sensory Evaluation of *Clitocybe nuda* Mushroom Extract in Liquid Whole Egg

by

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Abstract

The use of antimicrobial agents in the food industry is critical for the reduction and control of foodborne pathogens as part of food safety programs. The food industry is looking for new naturally occurring agents with bioactive properties including antimicrobial activity. Mushrooms have been known to contain many medicinal and bioactive agents responsible for their survival in the wild. *Clitocybe nuda* is an edible mushroom, located in Southeast Asia and the Middle East, used in many culinary styles of these regions. The purpose of this study is to perform a sensory evaluation of the mushroom extract to determine acceptable levels to panelists and to confirm antibacterial activity of the mushroom extract at these levels. The sensory evaluation was performed in liquid egg and tested for color, odor, texture, flavor, and overall acceptance at 0 (control), 10, and 20 mg per milliliter mushroom extract. The antibacterial testing was performed in liquid egg and TSB growth medium. The tests were performed on *Listeria monocytogenes* and *Salmonella* Typhimurium, Heidelberg, and Enteritidis at 4°C and 24°C. The sensory evaluation showed that the control sample scored the highest. The mushroom extract at 0, 10, 20, 60, and 90 mg/mL were tested for antibacterial efficiency. Greater antibacterial activity against *Listeria monocytogenes* than *Salmonella* spp was noted. Mushroom extract showed greater bacterial reduction in liquid eggs at 4°C.

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List of Abbreviations

AEB   American Egg Board

ANOVA   Analysis of Variance

BPB   Butterfield's Phosphate Buffer

BSA   Bismuth Sulfite Agar

CFSAN   Center for Food Safety and Applied Nutrition

CFU   Colony Forming Unit

CDC   US Center for Disease Control and Prevention

EO   Essential Oil

ETEC   Enterotoxigenic *Escherichia coli*

FDA   US Food and Drug Administration

HEA   Hektoen Enteric Agar

IFT   Institute of Food Technology

IRB   Institutional Review Board

M   Molarity

MOA   Modified Oxford Agar

NaOH   Sodium Hydroxide

OD   Optical Density

QDA   Quantitative Descriptive Analysis

SAS   Statistical Analysis Software

SPP   Species

TSA   Trypticase® Soy Agar

TSA + YE   Trypticase® Soy Agar plus Yeast Extract

TSB   Tryptic® Soy Broth

TSB+YE   Tryptic® Soy Broth plus Yeast Extract

TTB   Tetrathionate Broth

XLD   Xylose Lysine Deoxycholate

Chapter 1: INTRODUCTION

Background

The food industry has centralized production and is being distributed through nationally and internationally markets. The safety in food production, transportation, and storage has become a serious concern for the food industry. A single point of contamination anywhere in the food chain can lead to contaminated products to be shipped all over the world.

Every year, 1 in 6 Americans gets sick by consuming microbial contaminated foods or beverages (CDC 2014[b]). Forty eight million people become sick each year as well as 128,000 hospitalized and 3,000 deaths from foodborne illness. There are more than 250 different foodborne pathogens that have been identified and this makes it difficult to identify which one is responsible for each case of foodborne illness (CDC 2014[b]). Some of the most common pathogens are *Escherchia coli, Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella.* These bacteria are able to contaminate many different types of foods or beverages. In 2010, *Salmonella* Enteritidis was the cause of an outbreak in shelled eggs which spread across 11 states and lasted from May to November. Through the course of the outbreak, 1,939 cases were reported and 29 restaurants were found to be event clusters, a location where more than one case was reported. Wright County Egg in Iowa was found to be a supplier for 15 of the 29 restaurants and Hillandale Farms in Iowa was identified to be another possible source

of contamination. Both companies performed national voluntary recalls on their shelled eggs to bring an end to the outbreak (CDC 2010).

For food safety, the food industry uses a number of antimicrobials to inhibit the growth of pathogens. The globalization of the industry and changes in consumer opinions has created a desire for 'natural' or alternative antimicrobials. Countries around the world have different regulations on antimicrobial substances in foods and many American consumers are more interested in minimally processed and/or organic foods. Manufacturers in the food industry are looking for new naturally-occurring safe compounds.

Purpose of Study

The use of antimicrobials against pathogenic bacteria for food safety has long been a part of food manufacturer's safety protocols. Some consumers have begun to question the use of synthetic antimicrobials by the food industry. Therefore, the food industry is highly interested in finding new processing methods or naturally occurring materials that can provide antimicrobial properties. A number of fungi have been shown to have bioactive properties, including antimicrobial and antiviral activity. Some are being harvested for use in the medical and food industries. In the past few years, mushrooms have been identified as being a potential source of bioactive compounds.

Mushrooms of phyla *Basidiomycota* have shown promise for antibacterial properties. *Clitocybe nuda*, an edible mushroom, is one of the mushrooms with inhibitory activity toward several foodborne pathogenic bacteria. Bioactive compounds from *Clitocybe nuda* can have strong organoleptic properties that can affect the flavor or

aroma of the food. The purpose of this study is to perform a sensory evaluation to determine levels of *Clitocybe nuda* extract in liquid eggs that are acceptable to panelists, and to determine the effectiveness of *Clitocybe nuda* against *Salmonella* Typhimurium, *Salmonella* Heidelber*g*, *Salmonella* Enteritidis, and *Listeria monocytogenes*.

Significance of Study

There is a demand within the food industry to find new naturally-forming antimicrobial compounds. Many consumers desire minimally processed and natural food products from the industry. Foodborne pathogens, such as *Listeria monocytogenes* and *Salmonella* spp, are a growing concern for minimally processed foods due to inadequate temperature control. Improper refrigeration or incomplete heating of foods can allow bacteria to remain viable and multiply within the foods (Woteki and Kineman 2003). Eggs are an important ingredient in many foods and can transport bacteria into foods that may not receive proper heat treatments or may not have current antimicrobial compounds within them. The addition of antimicrobial agents to industrial liquid egg stock used in the formulation of food products can improve safety and value within the food industry.

Plants have been recognized as important sources of bioactive properties, including antibacterial, antifungal, and antitumor. The secondary metabolites naturally formed within plants are primarily responsible for these activities. Mushrooms have been shown to contain these compounds. *Clitocybe nuda* is an edible mushroom that has shown potential for antibacterial activity. The purpose of this study is to test

extracted *Clitocybe nuda* metabolites within liquid egg for sensory evaluation and

antibacterial activity towards *Listeria monocytogenes* and *Salmonella* spp.

Chapter 2: Literature Review

Foodborne Illness

"Food is the ideal vehicle for the dispersion of harmful agents because of the ability to mask the harmful agents by strong flavors, strong odors, various textures or intense colors (Cetin-Karaca 2011)." Food products are readily accessible to contamination during manufacturing processes. Food products are readily distributed over great distances which increases its chances of a single contamination impacting numerous people over a large area (Cetin-Karaca 2011). Every year, 1 in 6 Americans gets sick by consuming contaminated foods or beverages. Forty eight million people become sick each year, 128,000 are hospitalized and 3,000 deaths are attributed to foodborne illness. There are more than 250 different foodborne diseases have been identified (CDC 2014[B]). Animals have innumerable microorganisms found within them and on their hides. Though many are harmless and immaterial to humans, there are some that are pathogenic to humans. During slaughter, animal carcasses can become contaminated if exposed to the contents of the intestinal tract or if the hide is not properly removed. Fresh fruits and vegetables can also be contaminated by contact with microbes in the soil or if washed or irrigated with contaminated water. The water is most often contaminated with animal waste or human sewage (Cetin-Karaca 2011).

Foodborne illnesses from bacteria can occur due to three different methods: intoxication, toxicoinfection, and infection. Food intoxication occurs when bacteria

produce toxins within the food before consumption and the toxin causes the human illnesses. Notable examples of these types of bacteria are *Staphylococcus aureus* and *Clostridium botulinum*, which are able to produce enterotoxins and botulism toxin, respectively (CFSAN 2012). Toxicoinfection occurs when bacteria are ingested with food and the bacteria start to produce toxins after being ingested. Notable examples of this type of bacteria are *Vibrio cholera* and enterotoxigenic *Escherichia coli* (ETEC). The final method of foodborne illness, infection, is where the ingested bacteria are "invasive and can be harmful to the tissues and organs of the host (Bo 2012)."

Different diseases have different symptoms but all will typically begin within the gastrointestinal tract. Therefore nausea, vomiting, abdominal cramps, and diarrhea are common symptoms of many foodborne pathogens (CDC 2014[c]). Detecting a foodborne disease outbreak can be difficult because the microbes can be transmitted in multiple ways. Several pathogens, such as *Escherichia coli*, can be spread through contamination in foods, drinking water, swimming pools, or even person to person in some cases (CDC 2014[c]). Foods that are most at risk are raw or batched foods.   Meat, poultry, dairy, and shellfish are the most at risk products for contamination when raw. When foods are batched like eggs and milk, it only takes one contaminated source to infect the whole batch. Raw fruits and vegetables can also be high risk though washing can reduce the risk (CDC 2014[c]).

"An outbreak of foodborne illness occurs when a group of people consume the same contaminated food and two or more of them come down with the same illness (CDC 2014[c])." Many outbreaks are local occurrences typically picnics, pot-luck meals, or understaffed restaurants. However, greater numbers of foodborne pathogen

outbreaks are affecting large areas and more people. Companies distributing their products across larger geographical areas and sometimes under several different brand names (CDC 2014[c]). Sometimes, cases are not recognized as a foodborne illness outbreak but rather as a series of individual incidents. The greatest difficultly is identifying nation-wide outbreaks with only a few cases appearing in each state. Several factors such as consumer knowledge, disease surveillance by local health departments, incubation periods and severity of the diseases can affect the accuracy of reporting foodborne illnesses and outbreaks (Ghandi and Chikindas 2007).

A potential problem of growing concern in the food industry is antimicrobial resistant foodborne pathogens and their impact on the treatment of patients with antibiotics. This could be caused by the development of resistance of pathogens to sanitizing agents and antimicrobial agents used by the food industry. Serious debate surrounds the relationship between antibiotics used in animal husbandry. The widespread use of antibacterial agents, antibiotics, sanitizers, and disinfectants in food sanitation and their effect on antimicrobial resistance is being investigated. The ability of bacteria to adapt to adverse conditions is critical in their development of resistance (Ghandi and Chikindas 2007). This occurs when bacteria are exposed to sub-lethal levels of antimicrobial agents which can lead to adaptations in the bacteria and the development of resistance to higher levels of the antimicrobial and even the possibility of developing cross-resistances to other agents (Ghandi and Chikindas 2007). Some research has shown that the rotation of sanitizing agents can aid in minimizing the possibility of the development of resistant bacterial strains (Ghandi and Chikindas 2007).

There is a growing need for food preservatives and antimicrobials that can control food pathogens without having any adverse side effects to the food or the consumer. A primary reason for this is the increase in demand for fresh, minimally processed foods. Microbial contamination of eggs has important economic implications for the poultry industry. Two primary foodborne pathogens that are a concern for poultry and eggs are *Salmonella* spp and *Listeria monocytogenes* (Ghandi and Chikindas 2007; Jin and others 2008).

Salmonella

Salmonella spp, which are pathogenic to humans, are highly prevalent in all forms of poultry products. They are rod-shaped, Gram-negative, non-spore-forming, motile bacteria. Salmonellosis, disease caused by *Salmonella* infection, is a leading cause of foodborne illness in the United States, resulting in an estimated 1.4 million infections, more than 16,000 hospitalizations and nearly 600 deaths each year (Jin and others 2008). However, 4 serotypes can account for almost 50% of all human isolates reported in the United States, namely *Salmonella* Typhimurium (19%), *Salmonella* Enteritidis (14%), *Salmonella* Newport (9%) and *Salmonella* Javiana (5%) (Braden 2006). The mortality rate of Salmonellosis is typically less than 1%. However, *Salmonella* Enteritidis has a higher mortality rate, about 3.6%, among the elderly and the young, especially in hospitals and nursing homes (CFSAN 2012). *Salmonella* Typhimurium is the most common cause of human infection, though it is not the most prevalent of *Salmonella* spp. *Salmonella* Heidelberg is notable because from 1999 to 2005 the overall number of *Salmonella* cases decreased by 9%; however the

Salmonella Heidelberg cases increased by 20%. Though *Salmonella* Heidelberg is not a primary cause of Salmonellosis, it has a higher rate of resulting in critical infection in people compared to other *Salmonella* spp (Foley and others 2011).

The onset of symptoms occurs 6 to 72 h after ingestion. An infectious dose can be as low as only a few cells depending on the species and the health variables of the host. The typical symptoms of Salmonellosis are nausea, vomiting, abdominal cramps, diarrhea, fever and headaches. Symptoms will typically last 4 to 7 days depending on host variables, quantity of cells ingested, and the characteristics of the strain. Severe cases of Salmonellosis can lead to complications and serious health issues. Diarrhea and vomiting could lead to dehydration and an electrolyte imbalance in patients. This can be very dangerous to the young, elderly and immunocompromised people if they are not treated and it could lead to death in extreme cases. Another complication that may arise is an autoimmune response that causes arthritis several weeks after infection. The Center for Food Safety and Applied Nutrition (CFSAN) of the US FDA has stated that the autoimmune response can appear as joint inflammation, urethritis, uveitis, and/or conjunctivitis. *Salmonella* infections can also lead to blood poisoning and secondary infections in internal organs and joints if it is able to escape the gastrointestinal tract and spread to the rest of the body (CFSAN 2012).

Salmonella Enteritidis has become the primary cause of salmonellosis in the world, replacing *Salmonella* Typhimurium in 1990. *Salmonella* Enteritidis is typically found in eggs, although a number of *Salmonella* sterotypes commonly exist in poultry farms (Guard-Petter 2001). In laying houses, faecal and environmental sources can readily lead to contamination of eggs (Gast 2005). Entry of bacteria through cracks in

the egg shell can be a common cause of infection. Though *Salmonella* Enteritidis may pass through cracks in the egg shell but it is widely suspected that eggs are more often contaminated with *Salmonella* Enteritidis through vertical transmission from infected reproductive tracts (Guard-Petter 2001; AEB 2014[a]). Poultry infected with *Salmonella* Enteritidis, do not exhibit the common signs of infection, such as increased mortality, drastic weight loss and reduced egg production. Therefore, there are few signs to signal farmers of infected birds (Guard-Petter 2001).

Unpasteurized egg products or food products with raw eggs are considered at risk for *Salmonella.* In 1994, an outbreak of *Salmonella* Enteritidis occurred in Minnesota with 150 confirmed cases in September and October. The *Salmonella* Enteritidis contamination was determined to be from raw eggs in ice cream. The source of contamination was believed to be traced to sanitation of the tanker trailers due to back hauling of product (Hennessy and others 1996).

Eggs can be an inhospitable environment for bacteria such as *Salmonella* spp. Egg whites are rich in lysozyme and low in available iron, while the yolk contains antibodies. It is possible that some variants of *Salmonella* spp have become more adapted to surviving in the environment within an egg and that adapting to survive within eggs may cause these *Salmonella* variants to be more adapted to surviving within human hosts as well. *Salmonella* has been shown to be extremely versatile and able to adapt to new hardships and is the reason it remains a top cause of foodborne illness (Guard-Petter 2001). Most *Salmonella* spp require heating to 60 °C for 3.5 m in order to achieve a 3 log reduction of bacterial population according to the FDA (2001). Sheldon

showed that this time-temperature combination can produce a 9 log reduction (Sheldon 2005).

Listeria monocytogenes

      Listeria monocytogenes was first identified in 1924 and is a Gram-positive, rod-shaped, and facultative anaerobic. It is able to grow at temperatures below 1 °C, while many pathogens are unable to survive at similar temperatures. *Listeria monocytogenes* is also capable of surviving within acidified or high salt foods. This adaptability is a result of maintaining the fluidity of the cell membrane through holding lipids in a fluid crystalline state and adjusting the level of unsaturated fatty acids in the membrane as necessary to remain fluid (CFSAN 2012; Ghandi and Chikindas 2007).

      Listeria monocytogenes can be commonly found in water, soil, and decaying vegetable matter. Due to its prevalence, *Listeria monocytogenes* can readily enter into processing facility and a point of contamination can remain viable for years without proper sanitation (CDC 2013). *Listeria monocytogenes* could be found in virtually all food types. Uncooked vegetables and raw dairy products are commonly contaminated products. The largest outbreak for *Listeria monocytogenes* occurred in cantaloupe and it ranged across 28 states resulting in 147 illnesses and 33 deaths (CDC 2014[a]). Many prepared and ready-to-eat meats are susceptible to *Listeria monocytogenes* because it is capable of re-contaminating these products after cooking but before the product is packaged (CDC 2013; Ghandi and Chikindas 2007).

      Listeria monocytogenes is responsible for an estimated 1,600 illnesses and 260 deaths annually within the United States (CDC 2014[a]). Estimated mortality rate is 20%

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but survivors of infections will have long-term chronic suffering and susceptibility to new infections (McLauchlin and others 2004). The elderly, pregnant, newborn and immunocompromised populations are the most susceptible and most commonly infected by listeriosis (CDC 2013). People with diabetes, AIDS, or alcoholic liver disease are also at risk of infection (McLauchlin and others 2004).

Listeria monocytogenes infection typically leads to the disease listeriosis. Intra-uterine infection, meningitis, septicaemia, and central nervous system infection are the primary results of listeriosis (McLauchlin and others 2004). *Listeria monocytogenes* incubation time can vary from a few days to several months depending on mode and severity of infection (CFSAN 2012). The symptoms of a listeriosis vary depending on the health conditions of the host. A healthy person may only suffer mild symptoms or no symptoms at all. The typical symptoms are fever, muscle aches, nausea, and vomiting; some people might also experience diarrhea in unusual cases. Severe cases involving infections of the nervous system can cause headaches, stiff neck, confusion, loss of balance, and convulsions (CFSAN 2012). *Listeria monocytogenes* can also spread to infect a large number of other host tissues with the liver being a common site of secondary infection. In pregnant women, 25% of *Listeria monocytogenes* infections will result in still-born or abortion (McLauchlin and others 2004).

Listeria monocytogenes is of concern to liquid whole eggs because it capable of infecting the products in many conditions. It requires 62 °C for up to 108 s in order to reduce the bacterial population of *Listeria monocytogenes* by one log (Mackey and Bratchell 1989). In liquid whole eggs, traditional pasteurization of 60 °C for 3.5 m results in roughly 2.5 log reduction in *Listeria monocytogenes*. Ultra –pasteurization of

liquid whole eggs, temperature greater than 60 °C for less than 3.5 m, is capable of achieving up to 9 log reduction (Sheldon 2005).

Current trends in foods include minimally processed foods, and ready-to-eat foods. New processing techniques and globalization with imports of more ethnic foods are leading to more reliance on refrigeration and freezing for food safety. This has increased incidences of listeriosis in the past few years (Ghandi and Chikindas 2007). The FDA has a zero-tolerance policy for *Listeria monocytogenes* in ready-to-eat foods. Detection of *Listeria monocytogenes* at any level will classify a food as adulterated (Ghandi and Chikindas 2007). In 2002, *Listeria monocytogenes* was responsible for a multi-state outbreak in contaminated turkey deli meat. It resulted in 46 confirmed cases, seven deaths, and three fetal deaths over eight states (Ghandi and Chikindas 2007).

Listeria monocytogenes is a bacterium that has the ability to form biofilms. Microorganisms like *Listeria monocytogenes* can exist either as planktonic cells or as a community in a biofilm. Biofilms are able to attached to surfaces and enclose themselves within a matrix made up of primarily polysaccharide materials. Microbial biofilms show a decreased growth rate but an enhanced resistance to sanitizers and antimicrobial agents (Ghandi and Chikindas 2007). Biofilms are able to form on a number of surfaces including medical devices, system piping, and industrial equipment, as well as in numerous other areas of food processing facilities. They are most predominant in meat and dairy industries.

Biofilms are a concern because the bacteria can still spread and be transferred to food products while the source of the contamination remains difficult to eliminate. If a *Listeria monocytogenes* biofilm was formed within the lines of liquid whole egg

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processing, especially after the pasteurization step, then numerous batches can become contaminated. *Listeria monocytogenes* has been shown to either exist in monoculture biofilms or as part of a mixed culture with bacteria such as *Flavobacterium* (Ghandi and Chikindas 2007). *Listeria monocytogenes* in mixed culture biofilms are able to survive for longer periods of time than those in a monoculture biofilms. This is important because in food processing facilities there is an increased likelihood for mixed culture biofilms to form (Ghandi and Chikindas 2007). Mixed culture biofilms were found to be exponentially more difficult to sanitize those within biofilms. Exposure to 1,000 ppm free chlorine for 20 min was required to reduce *Listeria monocytogenes* biofilm by a two-log cycle. Normal *Listeria monocytogenes* was eradicated by an exposure to 10 ppm free chlorine for 30 s (Ghandi and Chikindas 2007).

Natural Antimicrobials

Food products are naturally perishable and require protection from spoilage during their preparation, storage, and distribution so that they may reach consumers within their shelf-life (Holley and Patel 2005). Since foods are now typically sold across various areas of the world far from its production facility, there is a need to be able to extend the stable shelf-life of products. Improvements in refrigeration and freezing methods have allowed for more international trade, but alone are not significant to ensure the preservation effectiveness of food products (Holley and Patel 2005). Due to greater consumer awareness of current synthetic chemical additives and growing public concern over the possible risks associated with health, there has been renewed interest in searching for more naturally produced safe alternatives. Researchers and food

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processors have been looking for new safe chemicals and substances with antimicrobial properties to use in the food industry (Cetin-Karaca 2011; Holley and Patel 2005).

This search has also led the researchers to review common products, such as plants, herbs, spices, and mushrooms, for new possible applications. Plants have become a prominent area of research over the past few years since plants produce materials and chemicals for their own defense against microbial invaders and these materials might be able to be applied to protect and preserve food products. Herbs and spices may have the capacity to do more than only flavor foods. The 'essential oils (EO)' of plants have shown they could be effective antimicrobials against a vast range of foodborne pathogens as well as spoilage microorganisms (Holley and Patel 2005). 'Essential oils' is a term that was coined in the 18th century by pharmacists to describe the extracts collected during processing and distillation of plants and spices. They were typically used as perfumes or flavorings since they contained volatile aromatic compounds responsible for the characteristics of the selected plant (Solgi and Ghorbanpour 2014).

When a plant must combat outside invasions, including parasitic microorganisms, they have secondary metabolites for protection. These metabolites may either be pre-formed within the host or produced due to stress situation. An example is when onions or garlic are sliced open, they will begin to enzymatically produce allicin. Examples of preformed secondary metabolites are phenolic compounds, glycosides, and alkaloids.

A drawback to use of some of these natural antimicrobials is their aromatic characteristics. There is a great demand for antimicrobial chemicals that will not alter the sensory characteristics of the food product. This may be a challenge for some

secondary metabolites. Researchers noted that concentrations necessary to achieve acceptable log reduction of notable pathogenic microbes for certain plant extracts were too high to maintain acceptable organoleptic characteristics in foods when screened by panelists (Cetin-Karaca 2011).

Metabolites

The number of different metabolites within living organisms is almost limitless. While many are primary metabolites, those required for necessary functions vital to the continued survival of the host, there are still many more that are needed for up keep and maintenance of the host; these are known as secondary metabolites. In 1999, Cowan and associates stated that over 12,000 secondary metabolites had been identified and they believed that this number is less than 10% of the total. Many have important roles to perform throughout the host. Terpenoids give plants their odors, quinones and tannins provide pigments to some plants, and many compounds imparting flavors to plants, both pleasant and unpleasant. However, most have some role to play in defense mechanisms of the host against predation by microorganisms, insects, and herbivores (Cowan 1999).

The phenolic compounds in essential oils typically exhibit the most antimicrobial activity (Holley and Patel 2005). Essential oils are effective even at low concentration. Gram-negative bacteria were more resistant to essential oils than Gram-positive bacteria. Phenolic compounds have several different modes of action as an antimicrobial. When phenolic compounds are at low concentrations, they affect enzyme activity and particularly affect energy production within pathogens. When at higher

concentration, phenolic compounds are able to denature proteins. The antimicrobial

activity of phenolic compounds is presumably a result of alterations to microbial cell

permeability due to changes in the cytoplasmic membrane. Other functions that can be

interrupted are electron transport, nutrient uptake, and nucleic acid synthesis (Holley

and Patel 2005; Tiwari and others 2009).

Flavones are a subset of phenolic compounds. They contain a single carbonyl

group with different side chain variations, such as a flavonol with one 3-hydroxyl group.

There are six subclasses of flavonoids: flavonols, flavones, isoflavones, anthocyanins,

flavanones, and flavonoids. Their antimicrobial activity may be due to the complex

reactions with extracellular and soluble proteins or the formation of complexes with

bacterial cell walls causing malfunctions. Lipophilic flavonoids may be reactive with

microbial membranes to cause disruption in functionality (Cowan 1999). Flavonoids are

the largest subset of phenolic compounds with over 9,000 compounds recognized.

Flavonoids have low molecular weights and contain 15 carbons in two aromatic rings

chained together. Flavonoids are typically found in high concentrations around the

outer layers of plants due to its biosynthesis associated with ultraviolet radiation

(Balasundram and others 2006; Cetin-Karaca 2011).

Quinones are another class of phenolic compounds that have great potential for

antimicrobial activity due to their ability to react irreversibly with the nucleophilic amino

acids in proteins, typically leading to inactivation of the protein and loss of functionality

(Cowan 1999). The possible targets of quinones are surface-exposed adhesins, cell

wall polypeptides, and membrane-bound enzymes as well as rendering substrates

unavailable (Cowan 1999).

Tannins are a group of polymeric phenolic substances capable of tanning leather, precipitating gelatin from solution, and primary source of astringency in many foods (Cowan 1999). Tannins have the highest molecular weights of phenolic compounds. The mode of action for tannins is similar to the activity of quinones. They have the ability to affect adhesins, enzymes, and transport proteins (Balasundram and others 2006; Cowan 1999).

Terpenoids are another type of secondary metabolites that are found in plants. Their structures are based on isoprenes with the primary form being called terpenes with a chemical formula of $C_{10}H_{16}$. Terpenoids also occur as hemiterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes (C_5, C_{15}, C_{20}, C_{30}, and C_{40}) (Cowan 1999). When an additional component is added to alter the compound, typically oxygen, they become terpenoids. How terpenes and terpenoids function as antimicrobials is still not fully understood but it is hypothesized that they disrupt the function of the cytoplasmic membrane. Terpenoids have been found to be highly effective in controlling *Listeria monocytogenes* (Cowan 1999).

Mushrooms

Many fungi have been shown to have many bioactive properties, including antibacterial, antioxidant, antitumor, and antiviral. However, higher order fungi, especially mushrooms, have been overlooked until the last few decades. Over the past two decades, research has shown that many mushroom species are showing similar antimicrobial metabolites that are found in many plants species. The reason is due to

the accumulation of various secondary metabolites, including phenolic compounds and terpenes (Suay and others 2000; Kalyoncu and others 2010).

There are several difficulties associated with using mushrooms as a natural source of bioactive compounds. Mushrooms are naturally seasonal and have slow growth rates with possibly years between harvests. A number of mushrooms also have issues with low production yields. These difficulties are now being "outweighed by the opportunity of finding new antibiotics with different structural types, as well as compounds with new modes of action (Suay and others 2000)." Novel mushrooms from around the world, such as *Clitocybe nuda*, are now being investigated for new compounds for bioactive properties (Suay and others 2000; Kalyoncu and others 2010).

Clitocybe nuda

Clitocybe nuda, also known as the Wood Blewit, is an edible mushroom in the Fungi kingdom from the phyla Basidiomycota, in the order Agaricales, and in the family of Tricholomataceae (Bo 2012; Wild Food Guide 2014). It was first named *Agaricus nudus* in 1790 and was transferred to the genus *Lepista* in 1871. Howard Bigelow and Alexander Smith proposed to change the name to *Clitocybe nuda*, though both names, *Clitocybe nuda* and *Lepista nuda*, are still accepted (First-nature 2011).

This mushroom is typically purple in color and grows on "decayed leaf litter in both coniferous and deciduous woodlands (Bo 2012)." They can also be found under hedgerows as well as urban areas. The *Clitocybe nuda* mushrooms can be found growing alone scattered across a large area or clustered close together. They typically grow from late summer through autumn and up to early winter. They can be found

fruiting all the way into December if it is a mild winter. They can be found in regions

around the world. They are most commonly found in Eastern Europe, the Middle East,

and Southeast Asia (First-nature 2011; Kuo 2010).

When it is young, *Clitocybe nuda* are blue or violet in color but as the mature they

begin to turn tan. The caps of the mushroom are typically 4-20 cm across and are

convex but may become nearly flat as they mature. The surface of the cap is smooth

with wavy margins and finely cracked across the center. The gills of the mushroom are

attached to the stem as well as being sinuated and crowded. The stem is 3-10 cm long

and 1-3 cm thick with a swollen base. The odor of *Clitocybe nuda* is fragrant similar to

aniseed and the taste is pleasant but not distinctive (First-nature 2011; Kuo 2010;

Bigelow and Smith 2014).

Clitocybe nuda is an edible mushroom that used in several different cuisines and

can be prepared several different ways. They are known to cause minor

gastrointestinal problems for some people if they are not cooked. Many consumers

enjoy *Clitocybe nuda* mushrooms when they are sautéed with pork or chicken. They

can also be paired with cheeses and pasta dishes or can be incorporated into omelets

and other egg dishes (First-nature 2011).

Clitocybe nuda has shown varying degrees of antimicrobial activity against

different pathogenic bacteria. These are an important part of the mushrooms defense

mechanism for surviving in its environment. Most notably, it is effective against

Salmonella spp, *Escherichia coli*, and *Staphylococcus aureus* (Mercan and others

2006)*.* The metabolites within the mushroom are one of the primary sources for the

mushrooms antimicrobial properties. The bioactive compounds of Clitocybe nuda are thermally stable at temperatures as high as 121 °C (Hou 2013).

Eggs Products

The 1970 Egg Products Inspection Act required that all commercial egg products must be subjected to pasteurization processes to reduce microbial load to an acceptable level (Jin and others 2008; AEB 2014[A]). The rate of contamination for *Salmonella* spp in eggs is 0.03%. The average level of infection for *Salmonella* is 10 CFU per contaminated egg. For Listeria monocytogenes, the rate of contamination in liquid whole eggs is about 5% of samples tested. For Listeria monocytogenes, the typical population in contaminated liquid whole egg samples is around 1.01 CFU/mL (Gast and Holt 2000; Rivoal and others 2010).

Quality attributes of liquid egg products are highly heat sensitive and it is important to use the minimum heat treatment possible while still providing proper safety in the final product (Jin and others 2008). Egg pasteurization was developed particularly to protect against *Salmonella*. Pasteurization causes no significant changes to the nutritional value of the egg however the flavor, color, and functional properties of the eggs may be affected. The functional properties of eggs are critical because eggs are used for their foaming, thickening, binding, and emulsifying properties (Ponce and others 1999). The heat of pasteurization can cause the formation or destruction of covalent bonds within the product leading to possible adverse changes and reducing product quality (Ponce and others 1999). The traditional pasteurization practice for all egg products is 60 °C for 3.5 m (FDA 2001). There is a very narrow margin of proper

time and temperature for pasteurizing that allows egg products to remain high quality and be free of microbial pathogens (Whiting and others 1997). There is evidence that coagulated parts of the liquid egg can have a lower rate of heat transfer than the remaining liquid sections. Whole eggs coagulate at 62.2 to 70 °C (144 to 158 °F) with egg whites coagulating first at temperatures between 62.2 to 65 °C and yolks coagulating between 65 and 70 °C (Egg Safety Center 2010). Consumers are recommended to cook all eggs to 160 °F; however servers and restaurants are required to heat eggs to 145 °F if for immediate use and 155 °F if for hot holding (HHS 2014; NRAEF 2012). *Salmonella* spp and Listeria monocytogenes can be destroyed at temperatures over 140 °F for a few minutes under normal conditions (Mackey and Bratchell 1989). Therefore, there is a risk that pathogens such as *Salmonella* Enteritidis may survive after pasteurization in these coagulated areas.

For traditional pasteurization, the typical shelf life of liquid whole eggs is from 5 days at 9 °C to 12 days at 2 °C. Liquid whole eggs can also undergo ultra-pasteurization which is heated to above 60 °C for a time less than 3.5 m. Ultra-pasteurization can allow liquid whole eggs to have a shelf-life between 3 and 6 months at 4 °C depending upon the quality of the eggs before pasteurization. Commercial liquid whole eggs, which are ultra-pasteurized, typically claim to have a 10 week shelf-life (Sheldon 2005).

The American Egg Board states that of the 76 billion eggs consumed in the US, almost 30% are in the form of egg products and further processed, such as dried, liquid, or frozen (AEB 2014[A]). Liquid eggs are an extremely valuable product due to being simple and versatile. It eliminates the need to break eggs and reduces the amount of

packaging and waste produced over shipping shelled eggs; reducing the cost of the product while increasing their overall value. Liquid eggs allow for easy rapid measuring of proper amounts by either volume or weight (AEB 2014[b]).

<u>Sensory Evaluation</u>

Sensory evaluation has been an important part of both research in academia and in the food industry during product development. Sensory evaluation has many different applications and can have several different meanings depending upon who is applying it.   It can become a challenge to properly perform a sensory evaluation in industry because the desire for specific answers may create question that can lead to biased answers. In general, researchers in academia have less chance of creating skewed bias in their studies and results (Sidel and Stone 1993). The Sensory Evaluation Division of the Institute of Food Technologists (IFT) defined sensory evaluation as a "scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing (Stone and Sidel 2004)."

Primary sensory attributes can also be known or perceived as appearance, odor, texture, and flavor. Appearance is an often underappreciated attribute. Appearance is one of the few attributes that consumers are able to use to impact their decision of purchasing a product, since they are often unable to taste or possibly even smell the product before purchasing. People use subtle clues from color, size, shape, surface texture, and clarity to determine the acceptability of the appearance (Meilgaard and others 1991).

Flavor is often considered one of the most important attributes in sensory evaluation. A simple definition of flavor is the interpretation of chemical reactions in the mouth and olfactory senses from tasting and smelling a food. Taste comes from clusters of epithelial cells called taste buds that are found throughout the bumps and grooves of the tongue. Taste buds are able to interpret the qualities of sweet, sour, bitter, and salty. A newly recognized taste called umami is also understood to be perceived by the tongue. More complicated aspects of flavor come from the addition of smell and its combination with taste (Lawless and Heymann 1998; Meilgaard and others 1991; Plattig 1988).

Odor or smell occurs when the aromatics from the food substance are perceived by the olfactory system. The high degree of diversity in food flavors is attributed to smell. Aromatics recognized by the olfactory receptors are either sniffed through the nose or enters into the nasal cavity up through the back of the mouth. Without the sense of smell, flavors will become dull and muted to only the basic flavors perceived by the mouth (Lawless and Heymann 1998; Meilgaard and others 1991).

Texture is the sensory attributes perceived by touch, sight, and hearing. Texture can be broken into three categories: mechanical characteristics, geometrical characteristics, and other characteristics. Geometrical characteristics are size, shape, and orientation of particulates in food. Other characteristics are the effects moisture and lipids in food. Mechanical characteristics are the largest category and are the effect of stress on food. Mechanical characteristics include hardness, cohesiveness, viscosity, elasticity, and adhesiveness. Texture is often associated with perception from

mouth manipulation or mastication. The sounds produced through mastication of a food can be considered texture for dry or crispy foods (Lawless and Heymann 1998).

There are three classes/types of testing in sensory evaluation: difference, descriptive, and acceptance testing. The testing of difference (also known as discrimination testing) is used to determine if there is a difference between samples overall or in a single characteristic. Triangle test or duo-trio test are typical tests used in difference testing. An example of difference testing use is when a company attempts to replicate or match a characteristic of anther product, such as replicating the flavor in an ice cream product to a low fat version. Paired comparison tests are discrimination tests used when testing two samples that differ only in one specific sensory attribute (Lawless and Heymann 1998; Meilgaard and others 1991).

Descriptive testing often involves identifying and/or ranking different characteristics of a product. They are ideal for shelf-life testing and determining how close new products are to currently accepted products, such as creating a sugar-free version of a product. They are highly effective but, it is a time consuming process that often requires training panelists to recognize flavors and specific characteristics as well as creating a specific jargon for each study. Therefore, there are difficulties in comparing results from multiple tests. Several commonly used descriptive tests are methods are flavor profile method, quantitative descriptive analysis (QDA) method, and free-choice profiling (Lawless and Heymann 1998; Meilgaard and others 1991).

Acceptance testing (also known as affective or preference testing) is primarily used to determine if a consumer enjoys a product, prefers it more than another product, or accepts it because of its sensory characteristics. Acceptance testing is an optimal

form of sensory testing because of its adaptability. It can be used in laboratory testing, centralized consumer testing, or home use testing. They can also be qualitative or quantitative. Qualitative affective testing works by measuring the subjective responses of panelists and a discussion of panelists' reactions. Quantitative affective testing uses large number of participants (100 – 400) to develop a consensus on preferences of sensory attributes (Lawless and Heymann 1998; Meilgaard and others 1991).

Chapter 3: Methods and Materails

Preparation of Mushroom Extract

Mushrooms

Dried *Clitocybe nuda* mushrooms were obtained from the National Chung Hsing University in Taiwan. The mushrooms were ground by a coffee grinder (General Electric, Fairfield, CT) for 45 s. Ground mushrooms were stored at room temperature in an airtight container (VWR, Radnor, PA) until extraction.

Preparation of Mushroom Extract

Mushroom extraction was performed by putting 25 g of ground *Clitocybe nuda* mushrooms in 600 mL of 95% ethyl alcohol (Pharmco-Aaper, Brookfield, CT) and stirred at 400 rpm for 24 h. The sample was vacuum filtered through a 5 μm filter paper (VWR, Radnor, PA) and, secondly, through a 0.45 μm filter (Fisherbrand, Pittsburgh, PA) in order to separate the infused alcohol. The ground mushrooms were extracted two more times using the same procedure as described above. The extracted solution was pooled for further processing.

The pooled alcohol solution was dried at 40 °C for 24 h to remove the alcohol. The dried extract was re-suspended in sterile deionized water and the solution was

centrifuged at 3,000 g. The supernatant was collected and dialyzed in a molecular weight cut off of 1,000 Dalton dialysis membrane. The membrane was submerged in sterile deionized water. The water was changed every 6 h for 3 times. Since the active compounds were smaller than 1,000 Da, the water was collected for further preparation.

The pooled mushroom extract solution was freeze dried in a Genesis SQ Super ES-55 freeze drier (SP Scientific, Warminster, PA). Dried samples were collected and stored at room temperature in a desiccator for use.

Test of Antibacterial Activity

Salmonella Heidelberg, Enteritidis, Typhimurium and *Listeria monocytogenes* were used in this study. *Salmonella* spp were used in equal portions.

Bacteria Preparation

Salmonella Heidelberg, Enteritidis, and Typhimurium were grown in Triptic[®] soy broth (TSB) (Becton, Dickinson and Company, Sparks, MD) separately for 16 hours. Then 0.5 mL of each serotype culture were combined together into a cocktail. The *Salmonella* cocktail was centrifuged at 4,500 g for 3 min. The supernatant was discarded and the pellet was washed twice with Butterfield's phosphate buffer (BPB) through centrifugation. The bacteria were re-suspended in BPB for use as stock bacterial suspension.

Listeria monocytogenes was grown in TSB plus 0.5% yeast extract (TSB+YE). The procedure used for *Listeria monocytogenes* preparation was the same as described above for *Salmonella*. The absorbances at OD_{640nm} of both bacterial suspensions were

measured for estimating the bacterial population. The designed bacterial inoculums were prepared through dilution from stock bacterial suspensions using BPB.

<u>Antibacterial Efficacy in Growth Medium and Liquid Whole Egg</u>

The mushroom extract was added at levels of 10, 20, 60 and 90 mg/mL to test antibacterial activity in TSB and liquid whole eggs to control *Salmonella* and *Listeria monocytogenes*. TSB and liquid whole eggs without mushroom extract were used as the control.

The antibacterial activity test was performed in bacterial growth media (TSB, and TSB plus yeast extract) and Rose Acre Farms liquid whole egg. The *Salmonella* cocktail and *Listeria monocytogenes* were used in this study. For the *Salmonella* test, TSB was used. For *Listeria monocytogenes* test, TSB plus 0.5% yeast extract were used. The mushroom extract was added to TSB at 0, 10, 20, 60, and 90 mg/ml. The pH of all samples was adjusted to 7 using 1M NaOH, and then samples were filtered through a 0.45 µm sterile membrane (VWR, Radnor, PA). *Salmonella* were inoculated in the samples at 10 and 100 CFU/mL for the initial inoculum. After inoculation, the samples were inoculated at 4 and 24 ^{o}C. The bacteria populations of samples were analyzed at day 1, 3 and 5 by spread-plating method onto TSA and TSA + 0.5% yeast extract plates. The plates were incubated at 37 °C for 24 hours and the bacterial colonies on the plates were recorded for analysis. For an antibacterial efficacy test in liquid whole eggs, the commercial liquid whole eggs from Rose Acre Farms were used. The test conditions and procedures were the same as used for testing in the growth media.

In order to confirm whether the mushroom extract was able to inhibit or kill the bacteria completely on the samples where no bacteria were recovered by spread-plating method, the enrichment process was employed to all tested samples.

Enrichment Testing

Enrichment testing was also employed on days 1, 3, and 5. For *Salmonella* testing, a sample was added to lactose broth (EMD Chemicals, Gibbstown, NJ) and incubated at 37 °C for 16 h for the pre-enrichment. One mL of pre-enrichment lactose broth was transferred to Tetrathionate Broth (TTB) (Neogen, Lansing, Michigan) and incubated at 37°C for 24 h. The enriched sample was streaked on *Xylose lysine deoxycholate (*XLD) (Becton, Dickinson and Company, Sparks, MD), Bismuth sulfate agar (BSA) (Becton, Dickinson and Company, Sparks, MD), and Hektoen Enteric agar plate (HEA) (EMD Chemicals, Gibbstown, NJ). Plates were incubated at 37°C for 24 h. The *Salmonella* growth was examined, and the results were recorded as positive or negative for *Salmonella*.

For *Listeria monocytogenes* examination, a sample was added to half strength TSB for 6 h at 24 °C for pre-enrichment. For enrichment, 1 mL of pre-enrichment broth was added to UVM Modified *Listeria* Enrichment Broth (Becton, Dickinson and Company, Sparks, MD) and incubated at 37 °C for 24 h. The enriched samples were streaked onto Modified Oxford medium plates (MOA) (VWR, Radnor, PA). Plates were incubated at 37 °C for 24 h and were examined for bacterial growth and the results were recorded as positive or negative for *Listeria monocytogenes*.

Sensory Evaluation

Approval from the Auburn University Institutional Review Board (IRB) was obtained to ensure the sensory evaluation was performed safely and ethically. The data obtained from the panelists had no information leading to the identity of the panelist. All score cards have been held securely since the completion of the sensory evaluation. This sensory evaluation was made up of experienced panelists, which included students, staff, and faculty in the Poultry Science Department. All participants had experience in participating in sensory evaluation panels from other studies within the department.

The sensory evaluation was conducted in the sensory kitchen of the Poultry Science Building. For the sensory evaluation, the egg samples were prepared by adding the designed level of mushroom extract in 475 mL of liquid whole egg. The liquid egg mixtures were blended for 45 s. The mixtures where baked in glass dishes at 350 °C for 20 to 25 m, when an internal temperature of 165 °F was reached. The eggs were cut into 1.5 cm squares and stored in a warming oven until given to panelists.

After a bench top evaluation by 6 trained panelists, working on this research project, it was determined that levels higher than 20 mg/mL were extremely unacceptable. Therefore, the amount of mushroom extract added to the liquid whole eggs was limited to levels of 20, 10 and 0 mg/mL for sensory evaluation.

Sensory evaluation was based on acceptance testing. The evaluation form contained a 6-point hedonic scale for color, odor, texture, flavor, and overall acceptability (Appendix 1). The scales of the score cards ranged from like extremely to

dislike extremely. Like extremely was given a code of 1 and dislike extremely was given a code of 6.

Panelists were asked to determine the acceptance of each sample. The samples were presented with randomly assigned three digit codes, which varied on each day of the study. In a randomly assigned order, panelists were provided a single sample at a time to score. The sensory evaluation forms were coded to correspond to the random codes given to the samples first presented. The process was performed on three days for a total of 51 evaluations.

Statistical Analysis

Statistical analysis was performed using SAS (SAS, 2014).   Analysis of variance (ANOVA) was performed with Tukey's multiple range test with comparison of means at 95% confidence level.

Chapter 4: Results and Discussion

Antimicrobial Activity Testing

Salmonella at 4 $^{\circ}$C in TSB medium

The mushroom extract was added in TSB at 10, 20, 60, and 90 mg/mL levels with inoculums at 10 and 100 CFU/mL of _Salmonella_ spp separately at 4 $^{\circ}$C up to 5 days for antimicrobial activity test. After the 5 days at 4 $^{\circ}$C, no bacterial reduction was found in any of the treatments.

Salmonella at 24 $^{\circ}$C in TSB medium

The results of mushroom extract tested against _Salmonella_ spp at 24 $^{\circ}$C in TSB can be seen in Table 1. A correlation between higher concentration of mushroom extract and higher bacterial log reduction can be determined. The 10 to 90 mg/mL treatments at 10 CFU/mL inoculated samples, the bacterial reductions ranged from 0 to 1.14 log on day 1, then the bacterial reduction reduced on day 3 and increased on day 5.

There was the same trend at the 100 CFU/mL initial bacterial inoculum samples. There was a higher bacterial reduction with higher initial inoculum samples than lower initial inoculum samples on day 1. The bacterial reductions were similar for both initial inoculum sets on day 3 and day 5. This may be the result of the inhibition of more

Table 1: Antibacterial Activity of Mushroom Extract against *Salmonella* at 24 °C in TSB medium

Inoculum Level	Treatment (mg/mL)	Log Reduction		
		Day 1	Day 3	Day 5
10 CFU/mL[a]	10	0.28	0.16	0.00
	20	0.22	0.00	0.54
	60	0.00	0.25	1.52
	90	1.14	0.40	1.20
100 CFU/mL[b]	10	0.16	0.59	0.36
	20	0.21	0.53	0.00
	60	1.91	0.08	1.21
	90	2.75	0.40	1.06

[a] The log bacterial population of the control on day 1, 3, and 5 were 5.65, 9.19, and 9.46
[b] The log bacterial population of the control on day 1, 3, and 5 were 7.39, 9.20, and 8.94

bacteria in the higher initial inoculum samples in the beginning which resulted in higher

bacterial reduction. After that, uninhibited bacteria grew back, as well as, the loss of

antibacterial efficiency of mushroom extract resulted in lower bacterial reduction on day

3 and day 5 (Table 1).

Salmonella at 4 ^{o}C in liquid whole eggs

The antibacterial activity against *Salmonella* spp was also tested in liquid whole

eggs. The levels of mushroom extract tested in liquid whole eggs were 0, 10, and 20

mg/mL, different from those tested in TSB, due to the negative effects of higher

mushroom extract in liquid whole eggs determined in the bench top sensory evaluation

test. In the liquid whole eggs, the antibacterial activity of 20 mg/mL mushroom extract

treated samples were significantly higher than those samples treated at 10 mg/mL in

both 10 and 100 CFU/mL *Salmonella* inoculated samples at 4 ^{o}C up to 5 days (Table 2).

On day 1, the bacterial reduction was highest in 20 mg/mL mushroom extract treated

samples at 2.87 and 2.92 log reductions in 10 and 100 CFU/mL inoculated samples,

respectively, and then it was reduced on day 3 and day 5. This may be the result of the

loss of the antibacterial activity of the mushroom extract over time and higher bacterial

populations of uninhibited bacterial growth. However, the mushroom extract still

possesses the antibacterial activity in 4 ^{o}C up to 5 days.

Salmonella at 24 ^{o}C in liquid whole eggs

The results of mushroom extract tested against *Salmonella* spp at 24 ^{o}C in liquid

whole eggs can be seen in Table 3. Bacterial reduction can only be seen on day 3.

Table 2: Antibacterial Activity of Mushroom Extract against *Salmonella* at 4 °C in Liquid Whole Eggs

Inoculum Level	Treatment (mg/mL)	Log Reduction Day 1	Day 3	Day 5
10 CFU/mL[a]	10	0.15	0.00	0.00
	20	2.87	1.35	1.53
100 CFU/mL[b]	10	0.27	0.37	0.00
	20	2.92	1.74	1.99

[a] The log bacterial population of the control on day 1, 3, and 5 were 6.10, 5.48, and 6.37
[b] The log bacterial population of the control on day 1, 3, and 5 were 6.09, 5.92, and 6.28

Table 3: Antibacterial Activity of Mushroom Extract against
Salmonella at 24 °C in Liquid Whole Eggs

Inoculum Level	Treatment (mg/mL)	Log Reduction		
		Day 1	Day 3	Day 5
10 CFU/mL[a]	10	0.00	0.20	0.00
	20	0.00	0.32	0.00
100 CFU/mL[b]	10	0.00	0.34	0.00
	20	0.00	0.18	0.00

[a] The log bacterial population of the control on day 1, 3, and 5 were 5.56, 9.38, and 9.15
[b] The log bacterial population of the control on day 1, 3, and 5 were 6.39, 9.46, and 9.04

There are only 0.20 and 0.32 log reductions on day 3 in the 10 and 20 mg/mL

mushroom extract treated samples with 10 CFU/mL *Salmonella* inoculated samples,

respectively. The 10 and 20 mg/mL mushroom extract treated samples with 100

CFU/mL bacteria inoculated had 0.34 and 0.18 log reductions, respectively, on day 3

(Table 3). This may be due to the higher growth rate of uninhibited bacteria at 24 ^{o}C or

possible ineffectiveness of the mushroom extract at these conditions.

Listeria monocytogenes at 4 ^{o}C in TSB + YE medium

The antibacterial activity was also tested against *Listeria monocytogenes*. The

mushroom extract was applied in TSB at 10, 20, 60, and 90 mg/mL levels with

inoculums at 10 and 100 CFU/mL of *Listeria monocytogenes* separately at 4 ^{o}C for up

to 5 days (Table 4). There was limited bacterial reduction in the extract samples on day

1 and day 3 at either *Listeria monocytogenes* inoculums under these conditions. The

bacterial reductions increased on day 5 in all samples; most notably in the 60 and 90

mg/mL mushroom extract treated samples.   The bacterial reductions were 1.60 and

1.86 log in 60 and 90 mg/mL treated with 10 CFU/mL bacterial inoculated samples,

respectively, and 1.01 log and 1.10 log in 100 CFU/mL inoculated samples (Table 4).

The bacterial populations in the control samples were around 3 log on day 5.

Listeria monocytogenes at 24 ^{o}C in TSB + YE medium

When the mushroom extract was tested at 24 ^{o}C, the antibacterial activities

began on day 1, were greatly reduced on day 3, and not seen on day 5 (Table 5). The

inoculum and concentration of the mushroom extract appears to affect the anti-*Listeria*

Table 4: Antibacterial Activity of Mushroom Extract against
Listeria monocytogenes at 4 °C in TSB + YE medium

Inoculum Level	Treatment (mg/mL)	Log Reduction		
		Day 1	Day 3	Day 5
10 CFU/mL[a]	10	0.00	0.17	0.29
	20	0.00	0.00	0.08
	60	0.00	0.00	1.60
	90	0.00	0.00	1.85
100 CFU/mL[b]	10	0.04	0.00	0.12
	20	0.00	0.00	0.14
	60	0.00	0.06	1.01
	90	0.23	0.79	1.10

[a] The log bacterial population of the control on day 1, 3, and 5 were 1.85, 2.27, and 4.01
[b] The log bacterial population of the control on day 1, 3, and 5 were 2.71, 3.30, and 3.58

Table 5: Antibacterial Activity of Mushroom Extract against
Listeria monocytogenes at 24 °C in TSB + YE medium

Inoculum Level	Treatment (mg/mL)	Log Reduction		
		Day 1	Day 3	Day 5
10 CFU/mL[a]	10	2.22	0.00	0.00
	20	2.72	0.08	0.00
	60	3.19	0.21	0.00
	90	4.24	0.33	0.00
100 CFU/mL[b]	10	0.12	0.22	0.00
	20	0.95	0.15	0.00
	60	2.98	0.01	0.00
	90	3.97	0.23	0.85

[a] The log bacterial population of the control on day 1, 3, and 5 were 8.23, 9.11, and 8.88
[b] The log bacterial population of the control on day 1, 3, and 5 were 8.37, 9.43, and 9.05

monocytogenes efficiency. The 10 to 90 mg/mL treatments at 10 CFU/mL inoculated samples, the bacterial reductions ranged from 2.22 to 4.24 log on day 1, then the bacterial reduction reduced on day 3 and without bacterial reduction on day 5. There is a similar trend in mushroom extract treated samples that were initially inoculated with 100 CFU/mL of *Listeria monocytogenes*. The bacterial reductions were lower in the 100 CFU/mL inoculated samples on day 1, compared to samples at 10 CFU/mL initial inoculum, since there were more bacteria in the inoculation, the growth was higher bacterial populations and lower bacterial reductions. Applying the same reasoning, there was little to no bacterial reductions observed on day 3 and day 5 (Table 5).

Listeria monocytogenes at 4 ^{o}C in liquid whole eggs

In the liquid whole eggs, at 4 ^{o}C, the mushroom extract did inhibit the bacterial growth and resulted in a 0.49 and 3.27 log reduction on day 1 in the 10 and 20 mg/mL mushroom extract treated samples, respectively (Table 6). When the inoculum was increased to 100 CFU/mL, the bacterial reductions were 0 and 3.50 log, at 10 and 20 mg/mL, respectively. On day 3 of the 100 CFU/mL treated samples, the bacterial reduction of the 20 mg/mL sample decreased to 1.90 log but the bacterial reduction increased to 2.47 log on day 5 (Table 6).

Listeria monocytogenes at 24 ^{o}C in liquid whole eggs

At 24 ^{o}C, in the liquid whole eggs treated at 10 or 20 mg/mL mushroom extract with 10 or 100 CFU/mL *Listeria monocytogenes* inoculated samples, the treatments

Table 6: Antibacterial Activity of Mushroom Extract against
Listeria monocytogenes at 4 °C in Liquid Whole Eggs

Inoculum Level	Treatment (mg/mL)	Log Reduction		
		Day 1	Day 3	Day 5
10 CFU/mL[a]	10	0.49	0.00	0.31
	20	3.27	1.59	2.19
100 CFU/mL[b]	10	0.00	0.00	0.47
	20	3.50	1.90	2.47

[a] The log bacterial population of the control on day 1, 3, and 5 were 6.07, 5.36, and 6.36
[b] The log bacterial population of the control on day 1, 3, and 5 were 5.95, 5.40, and 6.34

resulted in no bacterial reduction. Comparing the samples tested at 4 ^{o}C, it revealed

that the mushroom extract cannot completely inhibit the growth of *Listeria*

monocytogenes in all inoculated samples, and the uninhibited bacteria were able to

grow faster at 24 ^{o}C to the same level of the control samples and resulted in no

bacterial reductions. The purpose of testing at 24 ^{o}C was used to see whether the

mushroom extract could inhibit the bacterial growth completely and how long the

inhibition would last. Therefore, the mushroom extract appears to not inhibit the

bacterial growth completely at all tested temperature levels and inoculum levels.

Summary

   Clitocybe nuda extract showed limited antibacterial efficacy against *Listeria*

monocytogenes and *Salmonella* Typhimurium, Heidelberg, and Enteritidis. Many

mushrooms tested for antibacterial activity have shown limited activity against *Listeria*

monocytogenes and certain *Salmonella* spp. Rosa and others (2003) tested mushroom

species against bacteria and fungi species. Only two of the tested mushroom species,

Nothopanus hygrophanus and *Pycnoporus sanguineus,* were able to show activity

towards *Listeria monocytogenes* and a single mushroom species, *Irpex lacteus*, showed

activity toward *Salmonella* Typhimurium. Hou (2013) indicated that *Clitocybe nuda*

extract required a concentration above 20 mg/mL concentration to achieve a minimum

inhibitory concentration (MIC) of 50%. Barros and others (2008) found that *Lepista*

nuda, currently known as *Clitocybe nuda*, had limited activity towards all bacteria tested

except for *Bacillus* spp and *Staphylococcus aureas.* Dulger and others (2002)

confirmed that the *Clitocybe nuda* antibacterial activity was minimal towards *Salmonella*

spp. The results of this experiment are in agreement with the above mentioned

experiments.

Sensory Evaluation

Overview of Sensory Characteristics

The compiled sensory panel results are presented in Table 7. For the color characteristic, there was no significant difference (p≥0.05) between the control and the two mushroom extract treated samples. The control treatment received the lowest average score (2.18), which is equivalent to "Like moderately." The 10 mg/mL mushroom treatment also scored in the "Like moderately" range (2.28), while the 20mg/mL mushroom treatment received the highest average score (2.57), which equated to "Like slightly (Table 7)."

Color

To ensure that the sensory analysis was done in a consistent manner, the data was compared for each attribute on each day of testing. However, there was no significant difference between samples on the three days and the totals are shown in Table 8. There were no significant differences in panelist's scoring of color for each of the individual samples with the various treatments (10 mg/mL and 20 mg/mL) levels when compared to the control on the three days of evaluation (Table 8).

Table 7: Mushroom Extract Treated Liquid Whole Eggs by Experienced Panelists of All Sensory Attributes

Treatments	Color N = 51	Odor N = 51	Flavor N = 51	Texture N = 51	Overall N = 51
Control	$2.18 \pm 0.89*^{aA}$	2.16 ± 0.90^{aA}	2.04 ± 1.00^{aA}	2.37 ± 1.20^{aA}	2.24 ± 1.07^{aA}
10 mg/mL	2.28 ± 0.70^{aA}	3.29 ± 1.2^{bA}	3.24 ± 1.26^{bA}	2.90 ± 1.25^{abA}	3.14 ± 1.15^{bA}
20 mg/mL	2.57 ± 0.94^{aA}	3.57 ± 1.12^{bA}	3.82 ± 1.37^{cA}	$3.47 \quad 1.33^{bA}$	3.75 ± 1.15^{cA}

*   Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column ($p < 0.05$)

ABC – Means followed by the same character means no difference within a row ($p < 0.05$)

Table 8: Mushroom Extract Treated Liquid Whole Eggs Sensory Evaluation of Color

Treatment	Day 1 N= 19	Day 2 N=14	Day 3 N=18	Total N = 51
Control	2.37* ± 1.16 [a]	1.93 ± 0.62 [a]	2.17 ± 0.71 [a]	2.18 ± 0.89[a]
10 mg/mL	2.21 ± 0.71 [a]	2.29 ± 0.73 [a]	2.33 ± 0.69 [a]	2.28 ± 0.70[a]
20 mg/mL	2.58 ± 1.17 [a]	2.50 ± 0.86 [a]	2.61 ± 0.78 [a]	2.57 ± 0.94[a]

* Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column and row ($p < 0.05$)

Odor

There was a significant difference (p≥0.05) between the control and the two mushroom extract treated samples when the odor was evaluated by experienced panelists (Table 9). There was no significant difference (p≥0.05) found between the odors of the 10 mg/mL and 20 mg/mL mushroom extract treated samples. There was a regression in the values from the control which was evaluated in the "like moderately" range, while the 10 mg/mL sample was evaluated in the scale at the "like slightly" range with the 20 mg/mL sample being at the same level on the hedonic scale. There were no significant changes in panelist's scoring of each of the individual samples on any of the three days when evaluating odor (Table 9).

Flavor

There was a significant difference (p≥0.05) among the control and other two mushroom extract treated samples when evaluated for flavor on the three days of the sensory evaluation (Table 10). The control sample had the lowest mean score at 2.04 which equated to "Like moderately" and the daily means ranged from 1.71 and 2.26. The 10 mg/mL mushroom extract treated samples had a compiled mean of 3.24, equivalent to "like slightly" with daily means ranging between 3.47 and 3.07. The 20 mg/mL mushroom extract treated sample had a compiled mean of 3.82, equivalent to "dislike slightly" and its daily means ranged from 3.93 to 3.72. There was no significant difference in flavor between the 10 mg/mL and 20 mg/mL mushroom extract treated samples, while control samples were significantly different from the other two samples.

Table 9: Mushroom Extract Treated Liquid Whole Eggs Sensory Evaluation of Odor

Treatment	Day 1 N= 19	Day 2 N=14	Day 3 N=18	Total N = 51
Control	2.11* ± 1.05 [a]	2.00 ± 0.68 [a]	2.33 ± 0.91 [a]	2.16 ± 0.90[a]
10 mg/mL	3.16 ± 0.96 [b]	3.29 ± 1.20 [b]	3.44 ± 1.46 [b]	3.29 ± 1.2[b]
20 mg/mL	3.32 ± 1.20 [b]	3.71 ± 1.20 [b]	3.72 ± 0.96 [b]	3.57 ± 1.12[b]

* Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column and row ($p < 0.05$)

Table 10: Mushroom Extract Treated Liquid Whole Eggs Sensory Evaluation of Flavor

Treatment	Day 1 N= 19	Day 2 N=14	Day 3 N=18	Total N = 51
Control	2.26* ± 1.33 ^{aA}	1.71 ± 0.73 ^{aA}	2.06 ± 0.73 ^{aA}	2.04 ± 1.00^{aA}
10 mg/mL	3.47 ± 1.43 ^{bA}	3.07 ± 1.39 ^{bA}	3.11 ± 0.96 ^{bA}	3.24 ± 1.26^{bA}
20 mg/mL	3.84 ± 1.54 ^{bA}	3.93 ± 1.39 ^{bA}	3.72 ± 1.23 ^{bA}	3.82 ± 1.37^{cA}

* Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column (p<0.05)

ABC – Means followed by the same character means no difference within a row (p<0.05)

There were no significant changes in panelist's scoring of each of the individual samples on the three days when evaluating flavor (Table 10).

Texture

There was no significant difference (p≥0.05) between the control and the 20mg/mL mushroom extract treated sample for the combined total panelist scores when the panelist evaluated the texture characteristics (Table 11). There was no significant difference between the 10 mg/mL treatment and the other two treatments for total analysis. The 20 mg/mL extract treated sample was scored the highest at 3.47, equated to "Like slightly", while the control sample was the lowest scored 2.37 equal to "Like moderately." The means of the second and third day are similar to each other but day one resulted in no significant differences between the three samples. There was a notable decrease in the mean response in 20 mg/mL mushroom extract treated samples from day one to day two rising from 3.21 to 3.79; a change from like slightly to dislike slightly. These changes, as well as, the slight increase in approval for the 10 mg/mL mushroom extract treated sample from day one to day two are responsible for the shift in significance between the three samples. There were no significant changes in panelist's scoring of each of the individual samples on any of the three days when evaluating texture (Table 11).

Table 11: Mushroom Extract Treated Liquid Whole Eggs Sensory Evaluation of Texture

Treatment	Day 1 N= 19	Day 2 N=14	Day 3 N=18	Total N = 51
Control	2.37* ± 1.21 aA	2.36 ± 1.34 aA	2.39 ± 1.14 aA	2.37 ± 1.20aA
10 mg/mL	3.00 ± 1.37 aA	2.86 ± 1.35 abA	2.83 ± 1.10 abA	2.90 ± 1.25abA
20 mg/mL	3.21 ± 1.40 aA	3.79 ± 1.25 bA	3.50 ± 1.34 bA	3.47 ± 1.33bA

* Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column (p<0.05)

ABC – Means followed by the same character means no difference within a row (p<0.05)

<u>Overall Acceptance</u>

The overall acceptance of the samples when evaluated by the panelists was significantly different ($p \geq 0.05$) among all three samples (Table 12). The control sample was scored the highest at 2.24, equating to "like moderately", and the 20 mg/mL mushroom extract treated sample was scored the lowest at 3.75, equating to "dislike slightly." On day 1 and 2, there was a significant difference between the control and 20 mg/mL mushroom extract treated sample but there was no significant difference between the 10 mg/mL mushroom extract treated sample and the other two samples. On day 3, there were significant differences among all three samples. The change was due to the slight increase in acceptance of the 10 mg/mL sample between each of the days. There were no significant changes in panelist's scoring of each of the individual samples on any of the three days when evaluating the overall acceptance of samples (Table 12).

<u>Summary</u>

The panelists did not disapprove of the color change due to the addition the mushroom extract while the acceptance of texture did not appear to change significantly with from the control sample to the 10 mg/mL mushroom extract treated samples. Panelists appear to disapprove of the odor of mushroom extract treated samples, compared to the scoring of the control sample. Flavor appeared to be the most heavily impacted by the addition of the mushroom extract to samples and was nearly identical to the changes in overall acceptance. Adding additional ingredients, such as pepper or onions, to the preparation of eggs could alter characteristic profiles enough for the

Table 12: Mushroom Extract Treated Liquid Whole Eggs Sensory Evaluation of Overall Acceptance

Treatment	Day 1 N= 19	Day 2 N=14	Day 3 N=18	Total N = 51
Control	2.37* ± 1.38 aA	2.14 ± 1.03 aA	2.17 ± 0.71 aA	2.24 ± 1.07aA
10 mg/mL	3.32 ± 1.34 abA	3.14 ± 1.29 abA	2.94 ± 0.80 bA	3.14 ± 1.15bA
20 mg/mL	3.63 ± 1.26 bA	3.86 ± 1.23 bA	3.78 ± 1.00 cA	3.75 1.15cA

* Means values vary between 1 (Like extremely) and 6 (Dislike extremely)

abc – Means followed by the same character means no difference within a column (p<0.05)

ABC – Means followed by the same character means no difference within a row (p<0.05)

treated samples to be more appealing to panelists. The mushroom extract could be added to other food products with different results. The odor and flavor of the mushroom extract might be less pronounced in food products such as meat dishes, soups, or sauces.

Varying forms of mushrooms have been tested in food products over the past decade. In 2006, Eissa and Zohair tested ground *Saponaria officinalis* mushrooms in halawa, a traditional confection dessert of the Middle East, by replacing sesame paste at several levels. The researchers tested between 2 and 24 percent substitution with Hunter Color Lab method and sensory testing. The color testing resulted in darkened products, increased redness, and decreased yellowness as the concentration of ground mushroom increased. This browning in the color caused acceptance to decrease in color as the concentration of mushroom increased. The panelist scoring of texture and flavor characteristics were also lower in samples with the higher mushroom concentrations (Eissa and Zohair 2006).

Another experiment was performed to test mushrooms as possible meat filler. Cha and others (2014) tested white jelly mushrooms to replace pork ham in pork patties. Panelists were highly receptive to mushrooms replaced at the 10, 20, and 30% levels. They found all the attributes tested, except texture, to be acceptable. The 10% mushroom replacement scored the highest of all samples including the control sample (Cha and others 2012). Fan and associates (2006) tested mushroom flour created from mushroom polysaccharides to use in bread making. It was tested at up to a 12% replacement for regular flour, and panelists were unable to detect any significant differences in any characteristic except for color. The panelists scoring of the 3 and 6%

concentrations of mushroom polysaccharides showed no significant difference to the control. The 3% concentration sample was scored higher than the control in aroma, texture, and taste characteristics (Fan and others 2006).

Other studies performed with mushrooms in foods found that there are ways to use mushrooms successfully in food products and that adjustments to the egg samples could greatly improve the scoring of the panelists. The color change of the liquid whole eggs could be marginalized by the addition of ingredients of different colors resulting in the attention to be drawn away from the egg color. Aromatic compounds, garlic or herbs, could also improve the odor characteristics of the egg product. The addition of crisp vegetables, bell peppers and onions, could improve the texture of the product. The flavor could also be positively altered by the addition of any ingredients that improve the other characteristics. The improvement of the different sensory characteristics would improve the overall acceptance of the liquid whole egg products with mushroom extract. The addition of mushroom extract to food products with stronger sensory characteristics may lead to improved sensory acceptance of the mushroom extract in food products. Further research is required to determine the possibilities of sensory acceptance of the mushroom extracts in other food scenarios.

Chapter 5: Conclusion

Clitocybe nuda mushroom extract at the 10 mg/mL and 20 mg/mL levels showed marginal antibacterial activity towards *Listeria monocytogenes* and *Salmonella* Typhimurium, Heidelberg, and Enteritidis. The *Clitocybe nuda* mushroom extract had greater antibacterial activity towards *Listeria monocytogenes* as compared to the activity towards the *Salmonella* spp. The mushroom extract had a greater antibacterial activity in liquid whole egg than in growth media, TSB and TSB + YE.

Clitocybe nuda mushroom extract may not be highly effective as an antimicrobial; however it does have potential for use in hurdle techniques. *Clitocybe nuda's* heat resistance allows it to be added to liquid whole eggs before pasteurization without detrimental effects to its antimicrobial activity. This is a beneficial advantage to the application of *Clitocybe nuda* but research should be performed to determine how long the mushroom extract will have antimicrobial activity while combined with liquid whole eggs.

The sensory evaluation of mushroom extract treated liquid whole eggs samples compared to a control liquid whole egg sample revealed significant differences in panelist scoring of different sensory characteristics. The control sample was the highest scored for every sensory characteristic. However, the scoring of 10 mg/mL mushroom extract treated sample showed no significant difference to the control in color or texture characteristics. For 20 mg/mL mushroom extract treated sample, color was the only sensory characteristic with no significant difference to the control. However, flavor was

the only characteristic to be scored significantly different between the 10mg/mL sample and 20 mg/mL sample.

For liquid whole eggs, the addition of other ingredients during preparation may improve scoring of characteristics for the mushroom extract treated samples. Ingredients; such as bell peppers, onions, or meat; could improve or mask the characteristics of the mushroom extract.   The texture and color characteristics could become scored equally to or better than the control sample. The flavor and odor could have improved acceptability compared to the control. Further testing would be required to determine which additional ingredients may best improve panelist scoring.

References

[AEB] American Egg Board (2014[a]) Welcome to the American Egg Board - Egg Safety. http://www.aeb.org/food-manufacturers/egg-product-overview/37-history/96-egg-safety-history. Accessed 14 Oct 2014

[AEB] American Egg Board (2014[b]) Welcome to the American Egg Board - Frozen/Liquid. http://www.aeb.org/food-manufacturers/egg-products-specifications/44-whole-egg-types/134-frozen-liquid. Accessed 15 Oct 2014

Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry 99:191–203. doi: 10.1016/j.foodchem.2005.07.042

Bao HND, Ushio H, Ohshima T (2008) Antioxidative Activity and Antidiscoloration Efficacy of Ergothioneine in Mushroom (*Flammulina velutipes*) Extract Added to Beef and Fish Meats. Journal of Agricultural and Food Chemistry 56:10032–10040. doi: 10.1021/jf8017063

Barros L, Venturini BA, Baptista P, Estevinho LM, Ferreira ICFR (2008) Chemical Composition and Biological Properties of Portuguese Wild Mushrooms: A Comprehensive Study. Journal of Agricultural and Food Chemistry 56:3856–3862. doi: 10.1021/jf8003114

Bigelow, Smith (2014) California Fungi: *Clitocybe nuda.* In: MykoWeb. http://www.mykoweb.com/CAF/species/Clitocybe_nuda.html. Accessed 11 June 2014

Bo L (2012) Antibacterial activities of *Clitocybe nuda* extract on foodborne pathogens. Auburn University Master's Thesis

Braden CR (2006) *Salmonella enterica* Serotype Enteritidis and Eggs: A National Epidemic in the United States. Clin Infect Dis 43:512–517. doi: 10.1086/505973

[CDC] Center for Disease Control and Prevention (2010) CDC - Outbreak of Enteritidis Infections - December 2, 2010 - *Salmonella.* http://www.cdc.gov/salmonella/enteritidis/index.html. Accessed 13 Sep 2014

[CDC] Center for Disease Control and Prevention (2013) CDC - Risk & Prevention - Listeriosis. http://www.cdc.gov/listeria/risk.html. Accessed 15 Oct 2014

[CDC] Center for Disease Control and Prevention (2014[a]) Statistics | Listeriosis | CDC. http://www.cdc.gov/listeria/statistics.html. Accessed 15 Oct 2014

[CDC] Center for Disease Control and Prevention (2014[b]) CDC - 2011 Estimates of Foodborne Illness. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed 16 Oct 2014

[CDC] Center for Disease Control and Prevention (2014[c]) About Foodborne Illness | Food Safety | CDC. http://www.cdc.gov/foodsafety/facts.html. Accessed 16 Oct 2014

Center for Food Safety and Applied Nutrition (CFSAN) (2012) Bad Bug Book (Second Edition). In: U.S. Food and Drug Administration. http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm. Accessed 15 Oct 2014

Cetin-Karaca H (2011) Evaluation of Natural Antimicrobial Phenolic Compounds against Foodborne Pathogens. University of Kentucky Master's Theses

Cha M-H, Heo J-Y, Lee C, Lo YM, Moon B (2014) Quality and Sensory Characterization of White Jelly Mushroom (*Tremella fuciformis*) as a Meat Substitute in Pork Patty Formulation: White Jelly Mushroom in Pork Patty Formulation. Journal of Food Processing and Preservation 38:1018–1023. doi: 10.1111/jfpp.12058

Cowan MM (1999) Plant products as antimicrobial agents. Clinical microbiology reviews 12:564–582.

Dulger B, Ergul CC, Gucin F (2002) Antimicrobial activity of the macrofungus *Lepista nuda*. Fitoterapia 73:695–697. doi: 10.1016/S0367-326X(02)00233-2

Egg Safety Center (2010) Egg Safety: Cooking. http://eggsafety.org/consumers/egg-safety. Accessed 4 Dec 2014

Eissa HA, Zohair A (2006) Quality and safety of halawa modified with mushroom. Journal of the Science of Food and Agriculture 86:2551–2559. doi: 10.1002/jsfa.2645

Fan L, Zhang S, Yu L, Ma L (2007) Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. Food Chemistry 101:1158–1163. doi: 10.1016/j.foodchem.2006.03.017

[FDA] US Food and Drug Administration (2001) Safe Practices for Food Processes - Evaluation and Definition of Potentially Hazardous Foods - Chapter 4. Analysis of Microbial Hazards Related to Time/Temperature Control of Foods for Safety.

http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/u cm094147.htm. Accessed 2 Dec 2014

First-nature (2011) *Lepista (Clitocybe) nuda*, Wood Blewit identification. In: First nature. http://www.first-nature.com/fungi/lepista-nuda.php. Accessed 24 May 2014

Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC (2011) Population Dynamics of Salmonella enterica Serotypes in Commercial Egg and Poultry Production. Appl Environ Microbiol 77:4273–4279. doi: 10.1128/AEM.00598-11

Gandhi M, Chikindas ML (2007) *Listeria*: A foodborne pathogen that knows how to survive. International Journal of Food Microbiology 113:1–15. doi: 10.1016/j.ijfoodmicro.2006.07.008

Gast RK (2005) Bacterial Infection of Eggs. Food Safety Control in the Poultry Industry. CRC Press, Boca Raton, Florida, pp 1–20

Gast RK, Holt PS (2000) Influence of the level and location of contamination on the multiplication of Salmonella enteritidis at different storage temperatures in experimentally inoculated eggs. Poultry Science 79:559–563. doi: 10.1093/ps/79.4.559

Guard-Petter J (2001) The chicken, the egg and *Salmonella enteritidis*. Environmental Microbiology 3:421–430. doi: 10.1046/j.1462-2920.2001.00213.x

Hennessy TW, Hedberg CW, Slutsker L, White KE, Besser-Wiek JM, Moen ME, Feldman J, Coleman WW, Edmonson LM, MacDonald KL, Osterholm MT (1996) A National Outbreak of *Salmonella enteritidis* Infections from Ice Cream. New England Journal of Medicine 334:1281–1286. doi: 10.1056/NEJM199605163342001

[HHS] US Department of Health and Human Services (2014) Safe Minimum Cooking Temperatures. http://www.foodsafety.gov/keep/charts/mintemp.html. Accessed 4 Dec 2014

Holley RA, Patel D (2005) Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology 22:273–292. doi: 10.1016/j.fm.2004.08.006

Hou Z (2013) Antibacterial activities of secondary metabolites from *Clitocybe nuda*. Auburn University Master's Thesis

Jin T, Zhang H, Boyd G, Tang J (2008) Thermal resistance of *Salmonella enteritidis* and *Escherichia coli* K12 in liquid egg determined by thermal-death-time disks. Journal of Food Engineering 84:608–614. doi: 10.1016/j.jfoodeng.2007.06.026

Kalyoncu F, Oskay M, Sağlam H, Erdoğan TF, Tamer AÜ (2010) Antimicrobial and Antioxidant Activities of Mycelia of 10 Wild Mushroom Species. Journal of Medicinal Food 13:415–419. doi: 10.1089/jmf.2009.0090

Kuo M (2010) *Clitocybe nuda*. In: MushroomExpert.Com. http://www.mushroomexpert.com/clitocybe_nuda.html. Accessed 11 June 2014

Lawless HT, Heymann H (1998) Sensory evaluation of food. Principles and practices 227–253.

Lee D-U, Heinz V, Knorr D (1999) Evaluation of Processing Criteria for the High Pressure Treatment of Liquid Whole Egg: Rheological Study. LWT - Food Science and Technology 32:299–304. doi: 10.1006/fstl.1999.0553

Mackey BM., Bratchell N (1989) The heat resistance of Listeria monocytogenes. Letters in Applied Microbiology 9:89–94. doi: 10.1111/j.1472-765X.1989.tb00298.x
McLauchlin J, Mitchell RT, Smerdon WJ, Jewell K (2004) *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. International Journal of Food Microbiology 92:15–33. doi: 10.1016/S0168-1605(03)00326-X

Meilgaard MC, Carr BT, Civille GV (2006) Sensory Evaluation Techniques, Fourth Edition. CRC Press Boca Raton, Florida

Mercan N, Duru ME, Turkoglu A, Gezer K, Kivrak I, Turkoglu H (2006) Antioxidant and antimicrobial properties of ethanolic extract from *Lepista nuda* (Bull.) Cooke. Annals of microbiology 56:339–344.

[NRAEF] National Restaurant Association Educational Foundation (2012) Cooking Requirements for Specific Types of Food. ServSafe Manager Book

Plattig K.H. (1988) The Sense of Taste. Sensory Analysis of Foods, 2nd edn. Elsevier Applied Science, Essex, England, pp 1–22

Ponce E, Pla R, Sendra E, Guamis B, Mor-Mur M (1999) Destruction of *Salmonella* Enteritidis inoculated in liquid whole egg by high hydrostatic pressure: comparative study in selective and non-selective media. Food Microbiology 16:357–365. doi: 10.1006/fmic.1998.0248

Rivoal K, Quéguiner S, Boscher E, Bougeard S, Ermel G, Salvat G, Federighi M, Jugiau F, Protais J (2010) Detection of Listeria monocytogenes in raw and pasteurized liquid whole eggs and characterization by PFGE. International Journal of Food Microbiology 138:56–62. doi: 10.1016/j.ijfoodmicro.2010.01.013

Rosa LH, Machado KMG, Jacob CC, Capelari M, Rosa CA Carlos Leomar, Zani CL (2003) Screening of Brazilian basidiomycetes for antimicrobial activity. Memórias do Instituto Oswaldo Cruz 98:967–974. doi: 10.1590/S0074-02762003000700019

Sheldon BW (2005) Techniques for Reducing Pathogens in Eggs. Food Safety Control in the Poultry Industry. CRC Press, Boca Raton, Florida, pp 273–309

Sidel JL, Stone H (1993) The role of sensory evaluation in the food industry. Food Quality and Preference 4:65–73. doi: 10.1016/0950-3293(93)90314-V

Solgi M, Ghorbanpour M (2014) Application of Essential Oils and Their Biological Effects on Extending the Shelf-Life and Quality of Horticultural Crops. Trakia Journal of Sciences 12:199.

Stone H, Sidel JL (2004) 1 - Introduction to Sensory Evaluation. In: Stone H, Sidel JL (eds) Sensory Evaluation Practices (Third Edition). Academic Press, San Diego, pp 1–19

Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Dıez MT, Garcıa JB, del Val AG, Gorrochategui J, Hernandez P, Pelaez F, Vicente MF (2000) Screening of basidiomycetes for antimicrobial activities. Antonie van Leeuwenhoek 78:129–140.

Tiwari BK, Valdramidis VP, O' Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ (2009) Application of Natural Antimicrobials for Food Preservation. Journal of Agricultural and Food Chemistry 57:5987–6000. doi: 10.1021/jf900668n

Whiting RC, Buchanan RL (1997) Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. International Journal of Food Microbiology 36:111–125.

Wild Food Guide: Wood Blewit, (*Clitocybe nuda*), Including Recipes. In: Celtnet Recipes. http://www.celtnet.org.uk/recipes/ancient/wild-food-entry.php?term=Wood%20Blewit. Accessed 12 Aug 2014

Woteki CE, Kineman BD (2003) Challenges and Approaches to Reducing Foodborne Illness. Annual Review of Nutrition 23:315–344. doi: 10.1146/annurev.nutr.23.011702.073327

Appendices

Appendix A

Hedonic Attribute Scale

Egg Sensory Study

Please evaluate the egg sample for the following attributes: Before you taste the sample, evaluate the color and odor then taste the sample and evaluate the flavor and texture. You should eat some crackers and drink some water between samples to cleanse your palette.

<table>
<tr><td>

Color

☐ Like extremely

☐ Like moderately

☐ Like slightly

☐ Dislike slightly

☐ Dislike moderately

☐ Dislike extremely

</td><td>

Odor

☐ Like extremely

☐ Like moderately

☐ Like slightly

☐ Dislike slightly

☐ Dislike moderately

☐ Dislike extremely

</td></tr>
</table>

<table>
<tr><td>

Flavor

☐ Like extremely

☐ Like moderately

☐ Like slightly

☐ Dislike slightly

☐ Dislike moderately

☐ Dislike extremely

</td><td>

Texture

☐ Like extremely

☐ Like moderately

☐ Like slightly

☐ Dislike slightly

☐ Dislike moderately

☐ Dislike extremely

</td><td>

Overall

☐ Like extremely

☐ Like moderately

☐ Like slightly

☐ Dislike slightly

☐ Dislike moderately

☐ Dislike extremely

</td></tr>
</table>

Appendix B

Information Letter

AUBURN
UNIVERSITY

INFORMATION LETTER
for a Research Study entitled

"Sensory Evaluation of C. nuda Mushrooms in Liquid Eggs"

You are invited to participate in a research study to determine if the addition of mushroom metabolites to liquid eggs creates an acceptable flavor. The study is being conducted by Allen Gardner, graduate student in Auburn University Department of Food Science and Dr. Jean Weese, Professor in the Auburn University Department of Food Science. You were selected as a possible participant because you are faculty or staff of Poultry Science/ Food Science and you are age 19 or older.

What will be involved if you participate? If you decide to participate in this research study, you will be asked to taste samples of eggs and rank your reactions to different aspects of the egg samples. Your total time commitment will be approximately 15 minutes.

Are there any risks or discomforts? There are no known risks associated with participating in this study unless you have an allergy to eggs or mushrooms. IF YOU HAVE AN EGG OR MUSHROOM ALLERGY, DO NOT PARTICIPATE IN THE STUDY. You are responsible for any costs associated with medical treatment.

Are there any benefits to yourself or others? No benefits to panelist. If results of research is successful, there is a potential to improve the safety of the liquid egg supply.

Will you receive compensation for participating? No compensation is offered.

Are there any costs? There is no cost to the panelist to participate in the study.

If you change your mind about participating, you can withdraw at any time during the study. Your participation is completely voluntary. If you choose to withdraw, your data can be withdrawn as long as it is identifiable. Your decision about whether or not to participate or to stop participating will not jeopardize your future relations with Auburn University, the Department of Poultry Science or Food Science.

If you have any questions contact:

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