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Mitigating Mushroom Risks: Evaluating Cooking Practices for *Salmonella* Reduction in Dried Mushrooms

ABSTRACT

This study determines the inactivation of *Salmonella enterica* Stanley on dried mushrooms when prepared by one of three distinct consumer preparation techniques. Sliced and powdered wood ear mushrooms were inoculated with *Salmonella* Stanley and equilibrated for 2 days at 50% relative humidity. Samples were treated with different water temperatures and time parameters: 80°C water allowed too cool naturally or 100°C water and held for 10 min. Samples were removed at predetermined time intervals to determine log reductions. Sliced mushrooms soaked in room temperature water and subsequently treated with 100°C water had significantly higher log reductions at each time point ($P < 0.05$), yielding a 5-log reduction of *Salmonella* after 2 min. Sliced mushrooms that underwent treatment with 80°C water demonstrated a 5-log reduction of *Salmonella* after 10 min. A maximum of 4-log reduction of *Salmonella* was observed for powdered mushrooms treated with 80°C water. This study provides preparation recommendations to reduce *Salmonella* spp. populations in dried mushrooms.

INTRODUCTION

Wood ear mushrooms are characterized by their low-calorie content with high levels of protein and fiber. In addition, mushrooms boast substantial mineral content, such as iron and magnesium, alongside a noteworthy abundance of B vitamins. Beyond nutritional value, wood ear mushrooms have a history of medicinal application. Traditional Chinese medicine used these mushrooms for centuries to address diverse maladies, ranging from fevers and sore throats to colds *(18)*.

Wood ear mushrooms are grown on wood logs or in bags filled with sawdust at 22 to 32°C and are usually sold in fresh or dried forms. Drying mushrooms is an effective method of extending the product shelf life. Fresh mushrooms can have shelf lives as short as 1 to 3 days at ambient room temperatures *(8)* or up to 8 to 14 days at 2 to 3°C, if stored in modified or controlled atmospheres *(25)*. The common methods used in industry for drying fruits and vegetables involve convective air drying in tunnels or trays or vacuum, freeze, infrared, or microwave drying *(4, 21, 22, 23)*. Drying temperature is kept relatively low to maintain sensory

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properties of the foods; however, this limits the reduction of microbial load during the drying process *(2)*. Dried mushroom products are sold widely in retail grocery stores in plastic pouch packages. In some Asian traditional markets, it is common to see bulk quantities of dried mushrooms displayed in open air to be weighed by request and sold to customers *(1)*.

Generally, mushrooms, such as the wood ear variety, may be considered ready-to-eat in the United States, as they are frequently raw, according to the Mushroom Council *(19)*. Dried mushrooms are commonly used as ingredients or toppings in soups and cold desserts (e.g., snow fungus dessert soup), especially in Asian cuisines. In this instance, the product is rehydrated in water and applied directly onto food, sometimes without any additional cooking step *(17)*. In addition, there is also increasing popularity in using dried mushroom powders as coffee alternatives; the powders are steeped in warm water for an indefinite amount of time before consumption *(24)*. However, pathogens might potentially contaminate fresh mushrooms and therefore the final dried product. Mushrooms can be potentially contaminated during seeding or harvesting, as the substrates are made from raw agricultural materials *(31)*. This contamination risk is further exacerbated, as mushrooms are rarely subjected to postharvest washing to preserve quality and shelf life *(5, 29)*. Improperly sanitized processing equipment (i.e., slicer, dryer), as well as improper handling in storage, retail, and household settings (i.e., cross-contamination with raw meat and vegetables via a cutting board or utensils) can also introduce pathogens to the food product *(26)*. Once contaminated, pathogens can survive in these desiccated, low-water-activity storage conditions for over 6 months *(7, 10)*.

Contaminated dried and semidried mushrooms have been linked to numerous outbreaks and product recalls *(27, 28)*. In 2020, a multistate outbreak of *Salmonella enterica* Stanley in dried black fungus (i.e., wood ear mushroom) occurred, indicating that this food product can be a suitable vehicle for foodborne pathogens *(3, 27)*. However, there are currently limited guidelines to ensure safe preparation of dried mushroom products in household settings. Only one federal recommendation from a German institution exists, which outlines that dried mushrooms be heated to 80°C for at least 10 min *(9)*. Besides this, preparation methods suggested by online recipes or instructions found on product packaging remain inconsistent, invalidated, and vague for the reduction of foodborne pathogens *(Table 1)*. This raised a safety issue, as the common practice of rehydrating mushrooms prior to cooking can allow *Salmonella enterica* to grow to a considerable degree, potentially causing diseases *(1, 13)*. This study will address this issue by evaluating three different consumer preparation methods of dried (sliced and powdered) mushrooms on *Salmonella* Stanley survival. Time-temperature combinations were selected on the basis of available recipes and instructions listed on the product packaging. Results from this study will be useful to provide recommendations for best practices to consumers of these products and control for potential *Salmonella* contamination.

MATERIALS AND METHODS

Salmonella serotype

Salmonella Stanley H1256 (Department of Food Science and Technology, University of Georgia, Athens) was used in this study. The strain was maintained at −80°C on protectant beads in tryptic soy broth with glycerol (Microbank, Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada), and a working culture was maintained on tryptic soy agar (TSA; Difco, BD, Sparks, MD) plates at 4°C with monthly transfers to maintain viability. A single bead for the bacterial strain was removed and added to 10 ml of tryptic soy broth (Difco) and incubated at 37°C for 24 h. The broth culture was used to streak bacterial lawns of each strain on multiple plates of TSA. After incubation for 24 h at 37°C, the lawns of confluent growth were harvested by using a sterile L-spreader (VWR, Radnor, PA) and 1 ml of 0.1% peptone water (Hardy Diagnostic, Santa Maria, CA) to obtain highly concentrated inoculum (ca. 10 log CFU/ml).

Sample preparation and inoculation

Dried sliced mushrooms (dried black fungus slice; water activity [a_w] 0.56) were obtained from an online store (Amazon; product of People's Republic of China, distributed by Wei-Chuan USA, City of Industry, CA) and stored at room temperature until use. Prior to inoculation with *Salmonella* Stanley, the sliced dried mushrooms were autoclaved at 121°C for 15 min to inactivate relevant background microbial populations, which did not significantly alter the a_w (0.49). The background microbial populations of autoclaved wood ear mushroom were checked prior to inoculation, and no colonies were found on modified TSA with yeast extract plates that were incubated at 35°C for 24 h. Sliced dried mushrooms were aseptically weighed (180 g) and directly inoculated with *Salmonella* Stanley. An inoculum of 18 ml was sprayed onto sterile sliced mushrooms and homogenized manually in a sterile bag (Whirl-Pak, Nasco, Fort Atkinson, WI) for 2 min, after which the inoculated mushrooms were transferred into an aluminum foil tray and left to dry in the biosafety cabinet for 1 h. Next, 21 g of sliced mushrooms was aseptically separated and grinded until powdered form (mesh no. 25, 0.7 mm) using a spice grinder for 30 s and repeated three times at the same grinder setting (SG10 Electric Spice and Nut Grinder, Cuisinart, Stamford, CT). Both sliced and powdered mushrooms were stored in custom-designed relative humidity (RH) chambers equipped with water and silica beads columns, as well as a digital humidity sensor (SHT85, Sensirion AG, Stäfa, Switzerland) *(14)*. The RH chambers were set to the predetermined RH (30%), and

TABLE 1. Dried wood ear mushroom products in the market with preparation or cooking instructions on product packaging or product website, highlighting variety in preparation recommendations

Brand	Product description	Instructions on product packaging or website
Gong He	Black fungus	Soak in cold water for 6 to 8 h, rinse, and set aside. Fungus is good for cooking as a hot dish or cold dish.
Terra Dolce	Organic wood ear mushrooms	Soak in hot water for 15 min, rinse, and add to dish toward the end of cooking time to maintain crunchy firmness.
Asian Taste	Dried black fungus	Soak and wash with cold water, cook in 100°C boiling water. Stir-fry or stew fungus.
Orgnisulmte	Dried black fungus	Rehydrate black fungus using warm water for 30 min until tender, rinse with cold water.
Vigorous Mountains	Organic black fungus	Soak in warm water for 30 minutes or until soft, strain through a coffee filter to save liquid. Rinse with cold water and add to dishes for pan-frying, stir-frying, hot pot. Liquid can be added to dish to boost flavor.
Nuts.com	Dried wood ear mushrooms	Place amount into a bowl and cover with boiling water. Let soak for 12 to 15 min, stirring occasionally.
Spice Jungle	Wood ear mushrooms, shredded	Rinse product in cold, running water to remove debris. Put mushrooms in a bowl, cover with boiling water, and let soak for 25 min prior to draining.
Olive Nation	Shredded wood ear mushrooms	Soak in warm (not boiling) water for 20 to 30 min, then rinse. Repeat as needed until mushrooms are clean, then drain, and use.
Fortune	Dried black fungus (shredded)	Wash and soak in a bowl with warm water for 30 min. Remove and squeeze out excess water. Boil, braise, or stew until tender.

samples were equilibrated for 2 days. After equilibration in the RH chambers, the sliced and powdered mushrooms were removed from the chambers to measure the a_w of each product by using the a_w meter (TDL; AquaLab; Riverside, CA) to verify that the target aw was reached for the treatment (a_w 0.50 ± 0.02).

Treatments

To investigate the survival of *Salmonella* in dried mushrooms, three distinct treatments were evaluated, and three experimental replicates were conducted per treatment. The first treatment's parameters were inspired by the recommendations from the German Federal Institute for Risk Assessment, outlining that dried mushrooms be heated to 80°C for at least 10 min *(9)*. The second treatment was based on the packaging instructions of the dried mushrooms, which indicated "to pre-soak mushrooms for 30 minutes using cold water and cook thoroughly with 100°C water," with no cooking time being mentioned. The third treatment was selected to mimic steeping of mushroom powders for beverages. The powder to water ratio, as well as water temperature was selected on the basis of the recommendation made by the National Coffee Association *(20)*.

In treatment 1, 10 g of sliced inoculated mushrooms was aseptically weighed and combined with 90 ml of sterile distilled water at 80°C in a filter bag (Whirl-Pak). Water temperature was measured by using a Thermapen (THS-235-407, ThermoWorks, American Fork, Utah) to reach the target temperature before pouring water into the filter bag with dried mushrooms. The filter bag was twisted closed and left at room temperature (20 ± 2°C), and sampling was done at predetermined time intervals up to 10 min. In treatment

TABLE 2. Sample descriptions and treatment parameters

Mushroom product	Type of treatment	Time interval (min)
Sliced	Soak sliced mushrooms in 90 ml of sterile distilled water at 80°C	0, 2, 4, 6, 8, 10
	Soak sliced mushroom in 90 ml of sterile distilled water (30 min) + submerge in water bath at 100°C	
Powdered	Soak powdered mushrooms in 230 ml of sterile distilled water at 80°C	

2, 10 g of sliced mushrooms was soaked in ambient water (90 ml; 20 ± 2°C) for 30 min in a filter bag. The filter bag was submerged in a water bath held at 100°C for 10 min. Sampling was done directly after the ambient water soaking (before treating at 100°C) to evaluate the effect of presoaking and after treating at 100°C for a predetermined time interval. In treatment 3, powdered mushrooms (3 g) were weighed and combined with 230 ml of sterile distilled water at 80°C in a filter bag. The sample filter bag sat at room temperature, and sampling was conducted similar to treatment 1. In all treatments, multiple bags were prepared to assess different time intervals, and samples were analyzed at intervals of 2, 4, 6, 8, and 10 min. Temperatures of samples were recorded by using the Thermapen, and at the end of each time point, the filter bag was immediately cooled in ice water to halt pathogen inactivation. Each sample was serially diluted with sterile 0.1% peptone water and was homogenized for 30 s by using a stomacher (Seward, Weber Scientific, Hamilton, NJ). *Table 2* presents a summary of the treatments used in this study.

Enumeration of surviving bacteria

The samples were serially diluted with 0.1% peptone water and spread plated onto modified TSA. Garcia et al. tested several media recipes for the recovery of potentially injured *Salmonella* and found that TSA with yeast extract and sodium thiosulfate, modified for the selective and differential recovery of *Salmonella (9, 12, 15, 30)*, resulted in recovery that was better or similar than other recipes tested. All plates for the treatments were incubated at 35°C for 24 h.

Statistical analysis

Three replicates of each treatment were conducted. Bacterial counts were converted to log CFU per gram before statistical analysis. The data were analyzed using JMP Pro 16.2.0 software (JMP Statistical Discovery, Cary, NC). The analysis of variance and Tukey's pairwise comparisons were conducted to determine statistical significance.

RESULTS AND DISCUSSION

This study focused on the inactivation kinetics of *Salmonella* Stanley on sliced and powdered mushrooms under various rehydration thermal treatments. The rationale behind selecting the time-temperature combinations in the study was based on several considerations. At the time of the study, recommendations or best practices of dried mushroom preparations were not widely available or documented. Hence, this study based one of the rehydration methods on a recommendation made by the German Federal Institute for Risk Assessment that suggested heating dried mushrooms to 80°C for at least 10 min *(9)*. This method was tested against presoaking mushrooms with cold water, treating them in boiling (100°C) water, and allowing them to steep for 10 min, as suggested on the product packaging. The same instructions could also be found on a different brand's packaging of the same product (i.e., Asian Taste). Note that only a handful of dried wood ear mushroom products sold in the market are accompanied with cooking instructions. Some that do, often list varying instructions than the one used in this study (e.g., soak for 6 to 8 h in cold water or soak in hot water for 15 min; *Table 1*). Before experimentation, the aw and initial population of *Salmonella* in both sliced and powdered mushrooms were measured after exposure to RH of 50% for 2 days and are tabulated in *Table 3*.

Table 4 shows the *Salmonella* Stanley reductions for the three treatments across time intervals. At every sampling time point, sliced mushrooms that were soaked at ambient water for 30 min and cooked at 100°C exhibited significantly higher *Salmonella* reduction than other preparation methods, except at 10 min when the reduction was only significantly higher compared with that of powdered mushrooms treated at 80°C ($P < 0.05$). This method yielded >5 log CFU/g reductions of *Salmonella* Stanley populations after 2 min of treating at 100°C. Specifically, *Salmonella* populations were reduced by 7.3 ± 0.6 log CFU/g (initial population 8.6 ± 0.1 log CFU/g) after 10 min. Sliced mushrooms treated at 80°C water exhibited an eventual 5-log reduction after 10 min (5.2 ± 0.3 log CFU/g reduction). In contrast,

TABLE 3. The a_w and *Salmonella* Stanley population in sliced and dried wood ear mushroom before treatments

Type of mushroom	a_w (25°C)	*Salmonella* population (log CFU/g)
Sliced	0.509	8.6 ± 0.1
Powdered	0.511	8.1 ± 0.3

TABLE 4. Log reductions of *Salmonella* Stanley on dried wood ear mushroom samples when subjected to three consumer preparation treatments for different time intervals

Time	Sliced mushrooms soaked 30 min at ambient (20°C) + holding in 100°C water (log CFU/g survival)	Sliced mushrooms treated with 80°C water (log CFU/g survival)	Powdered (ground) mushrooms treated with 80°C water (log CFU/g survival)
2	6.7 ± 1.6^{Ba}	3.9 ± 0.9^{A}	3.5 ± 0.3^{A}
4	7.4 ± 0.4^{B}	4.0 ± 0.4^{A}	3.0 ± 0.5^{A}
6	7.6 ± 0.1^{Bb}	3.8 ± 0.5^{A}	3.5 ± 0.7^{A}
8	7.6 ± 0.1^{Bb}	4.1 ± 0.5^{A}	3.3 ± 0.8^{A}
10	7.3 ± 0.6^{Bb}	5.2 ± 0.3^{AB}	3.6 ± 1.5^{A}

[a]Least-squares means between treatments within the same time (sampling time) containing differing letters are statistically different from one another within a row ($P < 0.05$).

[b]Mean includes samples that showed total reduction below limit of detection of 0.9 log CFU/g.

treating powdered mushrooms in 80°C water resulted in a maximum 4-log reduction (3.6 ± 1.5 log CFU/g reduction) of *Salmonella* Stanley counts from an initial population of 8.1 log CFU/g after 10 min of treatment. *Salmonella* log reductions of sliced and powdered mushroom treated with 80°C water, though, were numerically different but were not statistically different from each other at each time point ($P > 0.05$). Also, presoaking sliced mushroom in ambient water (20°C) for 30 min yielded 8.7 ± 0.2 log CFU/g of *Salmonella*. This *Salmonella* count was not statistically different from the counts in the samples before treatments (8.6 ± 0.1; $P > 0.05$).

The results obtained from this study indicate that the preparation method, specifically temperature, was an influential factor in the survival of *Salmonella* in dried mushrooms. Treating dried mushrooms at higher temperatures (100°C) for 2 min promoted higher *Salmonella* reductions. Moreover, it is also crucial that the product is held at high temperatures (such as in stovetop cooking or using boiling water) to ensure effective inactivation. Conversely, *Salmonella* reduction might not be effectively achieved if dried mushrooms are only treated at sublethal temperatures (i.e., 80°C) and then left at room temperature without subsequent heating. This treatment mimicked soaking dried mushroom in a bowl of warm water, as is being widely suggested on the packaging instructions on multiple dried mushroom products in the market *(Table 1)*. The results suggest that this method may not be sufficient to eliminate *Salmonella* in the food product, emphasizing the need for additional cooking steps prior to consumption. Also, reconstituting dried mushrooms in warm or ambient water for extended periods has been shown to create an ideal environment for microbial growth *(4)*. Results of this study showed that presoaking mushrooms in ambient water for 30 min did not effectively reduced *Salmonella* counts in the product. In this case, cooking the food product at boiling temperature is a necessary step to effectively eliminate these microorganisms, thus significantly reducing the associated risk.

Multiple studies have demonstrated the survival of pathogens, including *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus*, to persist in dried mushrooms after dehydration or drying treatment and long-term storage *(13)*. After hot air drying (<45°C for 8 h) and 2-month storage, the mushrooms showed varying reductions for individual pathogens. *Salmonella* Typhimurium decreased by approximately 2 log, which would not be sufficient if the initial contamination level exceeded 10^2 CFU/g. Precautions are necessary, as the dried mushrooms could still retain contaminants.

A similar study showed that wood ear mushroom that was contaminated with *S. enterica* and *L. monocytogenes* (ca. 6.22 log CFU/g) showed 2.26 and 2.49 log CFU/g reductions, respectively, after 24-h dehydration at 60°C and 1-h drying at ambient temperature, whereas enoki mushrooms showed no reduction in the initial population of bacteria. This highlights the intrinsic variability of different mushroom types, such as the presence of antimicrobial compounds (e.g., polysaccharides, melanins, and polyphenols) in wood ear mushrooms *(7)*. In another study, ganodermadiol, a sterol in mushrooms, has been supported to have antiviral properties *(6)*. During 180-day storage at 25°C, the populations of *S. enterica* and *L. monocytogenes* reduced by approximately 1 to 2 log CFU/g to 1.79 and 1.78 log CFU/g, respectively. Once a pathogen survives in the food product, rehydrating the product at room temperature for a few hours before cooking introduces the risk of pathogens regrowing to unsafe levels *(13)*. Powdered wood ear mushrooms were used in this study to explore the growing trend of a blended mushroom beverage as a coffee alternative. Although mushroom "coffee" blends typically consist of other types of mushrooms (i.e., lion's mane, *Cordyceps*, reishi), we believe that the results found in this study would still provide beneficial data in determining the safety considerations of low-moisture mushroom powders.

The results of this study showed that grinding sliced mushroom to powdered form did not significantly impact the thermal inactivation kinetics of *Salmonella* Stanley ($P > 0.05$). In this study, mushroom powder was prepared by inoculating dried mushroom with *Salmonella* and grinding to fine powder by using a spice grinder. A study has indicated that the inoculation method used to prepare powdered foods might affect the survivability of *Salmonella* when challenged at high temperature. Specifically, the inoculation of almond meal and date paste prior to grinding of the samples increased the heat sensitivity of *Salmonella* as compared with the inoculation occurring post-grinding; however, the opposite was true for wheat flour *(16)*. This shows that the inoculation method might affect the survival of *Salmonella* differently in various low-moisture products. It was unknown how grinding would impact *Salmonella* heat resistance on mushrooms, but inoculating the sliced mushrooms prior to grinding allowed consistency with the other, nonground samples. Furthermore, particle size has been shown to impact the heating rate and inactivation of pathogens in dried food products, such as red pepper powder *(32)*. This study does not quantitatively measure the particle size of mushroom powder, but powder size was consistent by using the same amount of mushroom per batch and maintaining the duration of grinding. In addition, the results showed that steeping 3 g of inoculated powdered mushroom in 230 ml of 80°C water (a common powder to water ratio used for coffee and tea brewing) *(20)* and leaving them for 10 min to cool naturally did not result in a 5-log reduction of *Salmonella*, meaning these parameters could be insufficient in reducing *Salmonella* to safe levels. However, the differences in *Salmonella* reductions on powdered and sliced mushrooms after steeping in 80°water were not significantly different due to large standard deviations, though they were significantly less than sliced mushrooms held at 100°C after soaking in ambient water for 30 min. Hence, it is imperative to ensure that contamination risk is minimized early on during cultivation and processing of mushroom powders and to provide clear directions for heat treatments, so outbreaks related to these health foods do not occur in the future.

CONCLUSIONS

According to the data analysis, treating dried mushrooms in water at 100°C for several minutes (after presoaking the mushrooms for 30 min in ambient water) proves to be highly effective in reducing Salmonella. However, it was observed that in various cooking scenarios, the reduction of *Salmonella* was inconsistent when using temperatures lower than 100°C and not holding for specific time periods. Our findings shed light on the diverse effectiveness of thermal interventions in different dried wood ear mushrooms products. Properly implementing thermal treatments during mushroom processing can serve as a valuable strategy to mitigate pathogens and enhance food safety measures. These insights hold significance for retailers and consumers who may be using dried mushrooms in recipes, emphasizing the importance of adopting appropriate practices to safeguard consumer health and prevent potential foodborne illnesses.

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