Impact of FDA Produce Safety Standards on Mushroom Substrate Composting

Under the Food Safety Modernization Act (FSMA), mushroom producers must recognize potential food safety hazards in their operations and establish control measures to prevent them from occurring.

The Food Safety Modernization Act (FSMA) (P.L. 111-353) may be the most sweeping reform of our food safety laws in more than 70 years. The intent of the law is to change the way we as a country ensure the safety of our food supply. Instead of responding to an occurrence of contamination or an outbreak of food borne illness, the food industry is now challenged to proactively recognize potential food safety hazards in their operations and establish control measures to prevent them from occurring. The complexity of this shift in thinking is evident in the timing of the rule making process. Signed into law by President Obama on January 4, 2011, it took two years for FDA to begin releasing draft rules for public comment. Although each of the regulations that will come out of FSMA may affect the mushroom industry, the proposed rule "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" (Produce Safety Standards) (FDA 2013) will have the most direct impact on mushroom farmers.

The Produce Safety Standards rule includes requirements for controlling potential food safety hazards in areas where contamination is most likely to occur. These include:

1. Crop contact water use for irrigation and other agricultural purposes
2. Farm worker hygiene
3. Sanitation conditions affecting buildings, equipment and tools, and
4. Use of soil supplements containing animal manure.

FDA's preventative approach for ensuring the safety of produce is closely aligned with that of the Mushroom Good Agricultural Practices (MGAP) program, a set of farm food safety standards developed by the American Mushroom Institute and the Penn State Department of Food Science. MGAP core principles are:

1. Prevention of food safety hazards is favored over reliance on corrective actions after a problem has occurred
2. Mushrooms can become contaminated at any point between growing and shipping
3. The use of animal products in substrate, casing, or supplement preparation should be managed carefully to minimize the potential for microbial contamination of mushrooms
4. Worker hygiene and field sanitation practices play a critical role in minimizing the potential for microbial contamination of mushrooms, and
5. Water has the potential to be a source of contamination during mushroom growing and subsequent handling.

Perhaps at the top of the list of FDA's farm food safety issues is the use of raw and composted animal manures for conditioning and providing nutrients to agricultural soils. Animal manure is a likely source of many human pathogens, and crop contamination with animal feces has been linked to food borne outbreaks. The Produce Safety Standards propose to establish a 9 month interval between application of raw manure and harvesting. Any composting treatment that claims to reduce levels of human pathogens must be a scientifically valid, controlled, physical and/or chemical processes or composting processes that meets or exceeds specific microbial standards. Microbial limits for compost are linked to criteria on whether it can contact the crop and the amount of time between the treatment and harvesting. The draft rule states that compost suppliers will be required to document that microbial standards are achieved. On-farm composters will be required to document that time temperature conditions for a validated process are consistently achieved.

There are no known cases of food borne illness attributed to consumption of fresh mushrooms grown in North America. It is therefore likely that the industry use of horse and poultry manures in mushroom growth substrate formulations is safe. However, absence of evidence is not evidence of absence, and FDA's new regulatory approach will require the mushroom industry to provide scientific evidence that commercial composting is capable of eliminating human pathogens in raw manures. Research conducted in the Penn State Food Science Department provides some answers on the issue of the fate of human pathogens in mushroom growth substrate during phase II composting. The results from this research are published in August 2013 issue of the Journal of Food Protection in the article "Inactivation of Human Pathogens during Phase II Composting of Manure-Based Mushroom Growth Substrate" (Weil and others 2013) and are summarized below.

Phase II substrate validation study

A mixture of three pathogenic bacteria; *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. were grown to levels of approximately 10 8 colony forming units (CFU)/ml at the Penn State Food Safety Pilot Plant and then inoculated into post Phase I mushroom substrate. Inoculated substrate (2.5 kg) was placed into ventilated polypropylene bags and transported to the Penn State Mushroom Research Center (MRC) where they were placed in the center of standard wooden mushroom growing tray (24 X 24 X 12 in.). Uninoculated phase I substrate was filled around and above each bag until tray capacity was reached. The trays were subjected to a standard 6 day Phase II treatment which included a 2 hour pasteurization interval at 140 o F. Microbial levels for each of the three pathogens in inoculated substrate were determined before and after phase II. Levels of other microorganisms that may indicate contamination but are not necessarily pathogenic (*E. coli*, coliforms, *Enterobacteriacae*, total aerobic plate count) were determined in separate non-inoculated substrate before and after phase II.

Table 1 shows the populations of each of the pathogens and microbial indicators before and after phase II composting. No pathogens were found in any of the uninoculated samples. Initial levels of *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157:H7 after inoculation ranged from 7.2 to 8.1 log CFU/g. After phase II composting, none of the pathogens was detected by direct plating or by a more sensitive enrichment procedure. Levels for generic *E. coli*, coliforms, and *Enterobacteriacae* were all below detectable levels. In contrast, aerobic plate count populations, initially at nearly 9 log CFU/g, decreased by less than 2 log units during phase II composting. High aerobic plate count levels after phase II composting can be attributed to selective survival of heat resistant non-pathogenic thermophilic bacteria and fungi.

Survival of pathogens in inoculated Phase I substrate and indicator microorganisms in un-inoculated substrate was also studied at lower temperatures by holding vacuum sealed polypropylene bags in a temperature controlled water bath. Minimum times at which all three of the pathogens were not detectable by plating and enrichment at 120, 130, and 140oF were 36.0, 8.0, and 0.5 hours, respectively. *L. monocytogenes* and *E. coli* O157:H7 were more heat resistant than *Salmonella* spp., generic *E. coli*, total coliforms, and *Enterobacteriaceae*. This indicates that enumeration of indicator microorganism is not suitable for confirming complete pathogen destruction during phase II composting. Instead, determination of actual levels of *L. monocytogenes*, and/or *E. coli* O157:H7 would be necessary to verify the effectiveness of Phase II composting as a food safety preventative control.

Phase II substrate verification study

As a follow-up to the pathogen inoculation studies, a survey of post Phase II mushroom substrate was conducted at 12 Pennsylvania mushroom farms. Completed phase II substrate was sampled from three tunnels and nine bed farm growing rooms using compost sampling methods described in California Title 14, California Code of Regulations - Chapter 3.1, Article 7 - Composting Operations Regulatory Requirements and which are referenced in the Commodity Specific Food Safety Guidelines for the Production and Harvest of Leafy Greens (LGMA 2012).

At each farm, samples were taken from 12 locations distributed approximately evenly throughout the tunnel or growing room. Samples from two continuous tunnel systems were taken from the periphery of the substrate as it exited the tunnel. A third batch type tunnel was sampled at 12 evenly spaced locations as it was emptied by a front end loader. In growing rooms, samples were taken approximately 6 inches from the center front of a square to a depth of about 6 inches. At each sampling site, substrate samples (ca. 500 g) were taken by a gloved hand and filled into 55 oz. sterile Whirl-Pak™ bags leaving enough room for fold and twist tie sealing. The bags were packed into a cardboard box along with two frozen foam ice packs placed above and below the layers of bags to prevent changes in microbial levels. The boxes were overnight shipped by U.S. mail to a commercial laboratory for microbial analysis. Microbial populations for total aerobic plate count, coliforms, generic *E. coli* and enrichment procedures for absence or presence of *Listeria* spp. and *Salmonella* spp. were determined using established FDA or AOAC methods.

Results from the microbial survey are shown in Table 2. At each of the 12 farms, no detectable levels of coliforms, generic *E. coli*, *Salmonella* spp. or *Listeria* spp. were found in Phase II substrate samples. As expected, aerobic plate count levels, at each farm were high, ranging between 5.9 and 6.8 log CFU/g at bed farms and 7.1 and 8.4 CFU/g at tunnels. As previously discussed, these levels most likely represent beneficial thermophilic microorganisms that survive and grow at composting temperatures.

Conclusions and Recommendations

As the draft rule is now written, commercial suppliers of Phase II substrate will be required to provide verification data, through microbial testing, for the absence of human pathogens. Based on the studies described here, they should have no difficulty in meeting FDA standards. Although microbial testing will not be required for on-farm Phase II composting, growers will be required to collect time and temperature data to verify conformance to the validated process. Of course, this is something that growers are already doing, and should impose no new burden on their operations.

Beyond the FDA requirements, growers should consider conducting internal studies to find possible cold spots in their tunnels or beds. Temperature monitoring probes should be placed in these areas to assure that lethal composting temperatures are reached at all points in the substrate. A possible side benefit to this exercise would be more complete destruction of mushroom pests and fungal pathogens that affect crop yield and quality. Although substrate microbial testing by growers is not required by FDA, verification testing for *L. monocytogenes* on an annual basis or anytime major changes are made to the type and proportion of raw materials could be conducted. Because temperature monitoring and control equipment are important for obtaining consistent phase II heat treatments, they should be regularly maintained and calibrated to ensure that readings are accurate.

Another study conducted at the Penn State Mushroom Test Demonstration Facility (MTDF) showed that *Listeria* spp. can become established in the mushroom farm environment (Viswanath and others 2013). Therefore, where phase I composting is conducted at the same location as growing operations, MGAP standards for preventing cross-contamination should be strictly followed. These include keeping areas where straw bedded horse manure and poultry manure are received, stored, and handled physically separated from areas where mushrooms are grown and handled, casing soil ingredients are received and stored, and where mushrooms are shipped from the farm. The potential for cross contamination by water run-off, pests, wind, or movement of equipment and workers on the farm should periodically be evaluated and corrective adjustments made as necessary.

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Appendix

Table 1. Destruction of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* in inoculated substrate and indicator microorganisms in uninoculated substrate during phase II composting

	Microbial population (log CFU/g) a After Phase I	**Microbial population (log CFU/g) a After Phase II**
Inoculated pathogens		
L. monocytogenes	7.95 + 0.21	nd bc
E. coli O157:H7	7.95 + 0.21	nd bc
Salmonella spp.	7.40 + 0.28	nd bc
Indicator organisms		
E. coli	4.70 +/- 0.14	nd b
Total coliforms	5.10 +/- 0.28	nd b
Enterobacteriaceae	5.80 +/- 0.28	nd b
Aerobic plate count	8.75 +/- 0.35	6.90 +/- 0.43

a Mean + standard deviation of the mean population of bacteria. Populations were significantly different before and after phase II composting for all microorganisms ($\alpha = 0.05$). b Negative by the direct plating method (< 1.0 log CFU/g), c Negative by the enrichment method (< 1 cell/10g). nd = not detected.

Table 2. Populations of human pathogens and indicator microorganisms in substrate samples taken at commercial mushroom farms after Phase II composting.

		Microbial population (log CFU/g)				
Farm	**Phase II type**	**APC**	**Coliforms**	**Generic *E. coli***	***Salmonella* spp.**	***Listeria* spp.**
A	Tunnel	8.36	nd a	nd a	nd b	nd b

B	Tunnel	7.98	nd a	nd a	nd b	nd b
C	Tunnel	7.11	nd a	nd a	nd b	nd b
D	Bed	6.78	nd a	nd a	nd b	nd b
E	Bed	6.00	nd a	nd a	nd b	nd b
F	Bed	6.26	nd a	nd a	nd b	nd b
G	Bed	6.56	nd a	nd a	nd b	nd b
H	Bed	6.60	nd a	nd a	nd b	nd b
I	Bed	6.81	nd a	nd a	nd b	nd b
J	Bed	6.32	nd a	nd a	nd b	nd b
K	Bed	5.85	nd a	nd a	nd b	nd b
L	Bed	6.48	nd a	nd a	nd b	nd b

a none detected by direct plating method b none detected by enrichment method nd = not detected

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