

Microbial standards of commercially available produce

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Microbiology: Pre-Med

NIFS Intern Spring Session

14 04 2017

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Nomenclature

FDA - Food and Drug Administration
GAP - Good Agricultural Practices
HACCP - Hazard Analysis and Critical Control Points
ISS - International Space Station
MRM – Microbiological Risk Management
NACMCF - National Advisory Committee on Microbiological Criteria for Food
NASA – National Aeronautics and Space Administration
STEC – Shiga toxin-producing *Escherichia coli*
USDA - United States Department of Agriculture

Limits and guidelines are set on microbial counts in produce to protect the consumer. Different agencies make specifications, which constitute when a product becomes unsafe for human consumption. Producers design their procedures to comply with the limits, but they are responsible creating their own internal standards. The limits and guidelines are summarized here to be applied to assess the microbial safety of the NASA Veggie Program.

I. Introduction

In recent years, the amount of produce the average person consumes has increased, leading to an increased likelihood of foodborne pathogens. Microbial standards are put in place to minimize the chance for exposure to produce. However, it is not feasible to perform microbial analysis on every piece of produce. The solution is to do risk assessments of each step of manufacturing to determine the quantity and type of exposure. Plans are written to understand where the contamination risks are and how they can affect steps down the lines. In produce production, rules exist as to how frequently water and surface samples should be taken. Samples of the produce are also routinely taken to assess microbial contamination. These tests are used to predict the likelihood of contamination for the rest of the batch.

The Veggie system on the International Space Station is growing produce to be consumed by astronauts, making it necessary to evaluate what microbes could be potentially consumed and present on the ISS. It is impossible to run microbial evaluations on every sample, as this would destroy the limited amount of produce. Therefore, the growth process must be assessed to determine if the sanitization is effective. Analogs are run with ground control experiments to understand what microbes are present, and in what numbers.

II. Microbiological Criterion in Industry

The standards and guidelines for food safety are summarized in the Codex Alimentarius, an international agreement on food safety. The Codex standards are the baseline requirements for food trade, and each country is allowed to have more strict rules. The United States extends the guidelines in the Codex, and also follows the Hazard Analysis and Critical Control Points (HACCP) system. These combined together help producers set their microbiological criterion.

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The HACCP system is set up to identify potential risks, and assess how much damage they can cause, as well as how to control them. There are seven steps in developing a HACCP system, starting with a hazard analysis. A hazard analysis lists the potential hazards that are likely to cause harm, and an evaluation to determine the risk to the consumer. Critical control points are then determined, and are any point in the system where a potential hazard can be controlled and minimized. Critical limits are established based on standards, literature, experiments, and experts. Procedures are set up to monitor that the HACCP system is followed, and allows the producer to see where in the production contamination occurred. Corrective actions are also defined for violations of HACCP limits. Every test and step in the process is recorded so if contamination occurs, deviations from the normal levels are clear.¹

Although there are a series of systems in place to limit microbial contamination, the United States government does not have firm limits. They use the systems to set guidelines, but leave the limit creation up to the producer. The USDA created the National Advisory Committee on Microbiological Criteria for Food (NACMCF) to evaluate food safety standards and make internal additions to the Codex guidelines. The regulations set by the USDA are then enforced by the Department of Defense, who also create the consequences for violating microbial limits.

The Codex details how to create guidelines as a new producer, acceptable ranges, and the conduct of microbiological risk management (MRM)². The guidelines are reviewed frequently, and updated when needed. The current principles define each element risk analysis and incorporate aspects of the HACCP plan. The general principles are the following:

1. The primary objective is protecting the consumer's health
2. The MRM must take into account the complete food chain
3. The approach should be structured
4. MRM process should be transparent, documented, and consistent
5. Risk managers ensure consultations with interested parties
6. Risk managers ensure interactions with risk assessors
7. Risk managers take into account regionally different hazards and risk management options
8. Decisions are subject to review and revision when needed

The Codex also states that as soon as a contamination event is suspected, a risk manager must follow the MRM process to find the source of the food safety issue. The risk assessors, consumers, and industry must also be notified. A risk profile is a description of the issue and provides initial information and decisions. Risk profiles can occasionally give enough information to decide on a solution, and avoid further action. Actions can include establishing new standards and limits, requiring certifications, or developing new guides and trainings. Industry producers are also responsible for creating food safety systems, monitoring safety, and sampling produce.

A. Limits for various produce

The following are microbial guidelines set for a variety of different types of produce during the 2013-2015 NACMCF subcommittee meeting³. The meeting focused on setting microbiological criteria as indicators of when a process is out of control and when it becomes unsafe.

USDA uses a variety of standards and criteria to evaluate if there is proper microbial control over products. In general, a 5-log level of potential pathogens, such as *Staphylococcus aureus* or *Bacillus cereus*, is considered unsafe for human consumption. There is no general overarching rule on the acceptable level of microbes in the United States; companies with an interest in food safety make their own suitable criteria. Acceptable limits are determined in a case by case basis.⁴

Zones refer to how much contact a surface had with the finished product. Zone 1 has direct contact with the food, and is most sensitive to contamination. Zone 2 is non-food contact areas around the materials defined as zone 1, such as the floor or equipment in the general area. Contamination in zone 2 can end up in zone 1 through human or machine contact. Zone 3 is the area surrounding zone 2, such as a far corner of the room or a doorway, and can contaminate zone 2 through human or machine movement. Certain pathogens are more likely to show up in different zones, making it important to design a sampling procedure around the desired organism.⁵

Fruits and Vegetables (cut, frozen, or refrigerated)

Microorganism	Limit-unsafe	Action is limit exceeded
E. coli (generic)	100/g	Improve production hygiene, select better raw materials
E. coli (O157:H7 or other STEC)	Negative	Reject lot, investigate. Action depends on location, use of GAPs, and commodity
Listeria spp.	Negative in Zone 2 or 3	Investigate Zone 1, if present, reject lot. Action depends on location, use of GAPs, and commodity.
Salmonella	Negative	Reject lot, investigate. Action depends on location, use of GAPs, and commodity

Fruits and Vegetables (whole)

Microorganism	Limit-unsafe	Action is limit exceeded
E. coli (O157:H7 or other STEC)	Negative	Reject lot, investigate. Action depends on location, use of GAPs, and commodity
Listeria spp.	Negative in Zone 2 or 3	Investigate Zone 1 and finished product, use corrective action. Reject if in finished product
Salmonella	Negative in Zone 2 or 3	Investigate Zone 1 and finished product, use corrective action. If present in product, reject lot and use corrective action. Action depends on location, use of GAPs, and commodity.

Packaged Salads and Leafy Greens

Microorganism	Limit-unsafe	Action is limit exceeded
E. coli (generic)	100/g	Improve production hygiene, select better raw materials
E. coli (O157:H7 or other STEC)	Negative	Reject lot, investigate.
Listeria spp.	Negative in Zone 2 or 3	Investigate Zone 1 and finished product, if present, reject lot.
Salmonella	Negative	Reject lot, investigate, use corrective action

Vegetable Sprouts

Microorganism	Limit-unsafe	Action is limit exceeded
E. coli (generic)	10 ³ /g	Investigate, use corrective action
E. coli (O157:H7 or other STEC)	Negative in 2 50g analytical units	Reject lot, investigate, use corrective action
E. coli (O157:H7 or other STEC) in spent irrigation water	Negative in 2 100g analytical units	Reject lot, investigate, use corrective action
Listeria spp.	Negative in Zone 2 or 3	Investigate Zone 1 and finished product, use corrective action. Reject if in finished product
Listeria spp. (product)	Negative in 2 250 g analytical units	Reject lot, investigate, use corrective action. Note: 250g unit can be five 50g samples
Salmonella	Negative in thirty 50g analytic units	Reject lot and use corrective action.
Salmonella (spent irrigation water)	Negative in two 375 gal analytical units	Reject lot, investigate, use corrective action

III. Sanitizing produce and equipment

Equipment that regularly has contact with produce is highly prone to microbial contamination, which can in turn be passed to the produce. Workers can contaminate produce, and worker negligence can damage produce, causing it to become more susceptible to microbial growth. Water quality, light, temperature, and carbon dioxide concentration also can contribute to microbial growth in produce or on equipment.

There are different types of sanitizers, and each works better at targeting different organisms. Chlorine-based sanitizers are used most frequently, and work well against certain bacteria and yeasts on hard surfaces. Iodine works well on yeasts, molds, and vegetative bacteria, and generally have a broader range, however hardly effect spores. Ammonium sanitizers are effective at removing molds and *Salmonella*, but do not work against clinical bacteria and coliforms. Multiple products can and should be used to clean equipment to prevent cross-adaptation of bacteria and biofilm development. Managing biofilm growth is especially important, as many common human pathogens form biofilms, making them more resistant to sanitizers.

Produce is frequently cleaned by dipping into chlorine, however this only reduces bacteria populations by 1 or 2 logs, and has also been found to only reduce colony counts for the first few days before returning to the same levels as untreated produce.⁶ Studies have been done using chlorine dioxide gas to sanitize lettuce leaves. The lettuce was inoculated with *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* and then treating the leaves with chlorine dioxide gas for 30 minutes, 1 hour, and 3 hours. After treatment, microbial counts were done, and the 30 minute treatment had a 3.4-log reduction in *E. coli*, a 4.3-log reduction in *Salmonella*, and a 5.0-log reduction in *Listeria*. The 1 hour treatment had 4.4, 5.3, and 5.2 log reduction respectively, and the reduction in the 3 hour treatments was 6.9-log, 5.4-log, and 5.4-log. The gas did not harm the appearance of the produce, and worked as effectively on damaged plants.⁷

In the enclosed environment of the International Space Station, sanitizers like hypochlorite and hydrogen peroxide can't be used, as they are known to produce free radicals. A safe and biodegradable solution called PRO-



SAN, a citric acid based cleanser, was created by Microcide, Inc. for NASA to use on fresh produce in space. PRO-SAN is available in powder tablets and solutions, and can be used to create sanitizing wipes for produce. The wiped produce retains its flavor, but kills *E. coli*, *salmonella*, *listeria*, *cholera*, *shigella*, *staphylococcus*, and *streptococcus* at a 99.999% efficiency rate.⁸ NASA has recently been using this technology to clean cabbage and lettuce grown in Veggie pillows on the ISS, allowing the astronauts to consume fresh produce.

Figure 1 Astronaut Peggy Whitson places leaves of Tokyo Bekana Chinese cabbage between PRO-SAN soaked wipes, before astronauts consume the harvest. Credit: NASA TV

Conclusion

These limits and guidelines can be used to assess if a new producer's process is microbial safe for producing food. The producer is responsible for taking into account the recommendations from different agencies, both inside the United States and globally. These standards protect the consumer from disease and sickness. Knowing what microorganisms to expect, and in what numbers, can aid in choosing the best sanitization methods. The Veggie program at NASA can use these guidelines to ensure the space biology program follows and exceeds the microbial standards of producers on Earth. Understanding what microbial counts are unsafe, and when microbial growth occurs can prevent potential contamination to new planetary systems.

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