Health Canada Santé Canada

Food Directorate

Guidance for Industry:

Sample Collection and Testing for Sprouts and Spent Irrigation Water

Food Directorate
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This document is available at:
http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/sprout_water_testing_analyse_pousses_eau_e.html

Introduction

This guidance document is part of Health Canada's *Policy to Manage Health Risk Associated with the Consumption of Sprouted Seeds and Beans*[1]. It is to be used by stakeholders, sprout producers, private laboratories and government personnel when doing an analysis for the detection of *Salmonella* species (spp.) and *Escherichia coli* (*E. coli*) O157:H7 in sprouts or spent irrigation water.

Testing of Spent Irrigation Water

Procedures are provided for testing spent irrigation water and sprouts. Health Canada recommends that processors should regularly test, as a minimum, spent irrigation water.

Spent irrigation water that has flowed over and through sprouts is a good indicator of the types of microorganisms in the sprouts themselves. Microorganisms found in spent irrigation water is expected to be fairly uniform. Thus, sampling procedures for the spent irrigation water are relatively simple. Furthermore, water can be used directly in the test procedures described here. The only potential disadvantage of testing spent irrigation water is that the level of microorganisms recovered in spent irrigation water is about one log (10 times) less than the level in sprouts. If pathogens are present in sprouts at very low levels, it is possible that they may not be detected in the spent irrigation water, but could be recovered in sprouts if sprouts were tested.

However, testing the sprouts themselves has several significant disadvantages. First, a number of sprout samples must be taken from different locations in the drum or trays to ensure that the sample collected is representative of the batch. Furthermore, additional preparation (e.g., selecting representative sample units for analyses, blending or stomaching, and allowing sprout particles to settle out) is required when testing sprouts. Finally, the need to wait for analytical results on the sprouts will delay the delivery of the final product to the market. Each additional step in any procedure (sampling or testing) could introduce contamination.

Consequently, sprouts should not be tested in place of spent irrigation water unless production methods make it impossible to test spent irrigation water.

Note: The recommendation to test spent irrigation water does not preclude additional testing of sprouts (either sprouts collected during production or finished product).

Sample collection

[1] http://www.hc-sc.gc.ca/fn-an/legislation/pol/sprouts_pol_pousses_e.html

Sample collection should be done on site by personnel that have been trained to collect representative samples aseptically. Aseptic sampling procedures are described below.

Testing Facility

Health Canada recommends that all microbial testing for pathogens be conducted in an external, certified, independent laboratory that should meet several key criteria. First, the laboratory should be physically separated from the food production facility to prevent cross-contamination from test materials. This is especially important since the materials used in the enrichment step required before testing and the positive controls can contain pathogens and, if not properly handled, may contaminate sprouts. Second, the laboratory should be staffed by personnel with training and experience in analytical microbiology techniques to ensure that tests are performed correctly and that all appropriate safety precautions, including appropriate waste disposal, are followed. Third, the laboratory should have appropriate resources and be able to demonstrate that they follow a quality management system. If the microbial analysis is done by the sprout producer, the laboratory facilities, personnel, and management system should also meet all these criteria to ensure that testing is reliable and does not create food safety hazards.

The following factors should be considered in determining when and how to sample.

When to Sample

Pathogens are most likely to be present at detectable levels 48 hours after the start of the sprouting process. Levels will not necessarily increase after 48 hours and may decline slightly. Thus, collecting samples for testing can be done as early as 48 hours after the start of sprouting. If seeds are pre-soaked (e.g., soaked in water for a short time and then transferred to growing units for sprouting), include the pre-soak time.

Early results will allow a sprout producer to take corrective actions sooner, thus minimizing the potential for one contaminated batch of sprouts to contaminate other production batches. Sampling and testing 48 hours after the start of sprouting will also minimize the time and resources spent on a batch of sprouts if a presumptive positive is found. If a producer's action plan includes running confirmatory tests on a presumptive positive before discarding product, testing earlier rather than later allows more time to run additional tests.

How to Sample

Aseptic procedures are critical in order to avoid contaminating the sample during sample collection, storage and transportation to the laboratory. Aseptic sampling procedures, as described below, should be part of a producer's sample collection plan.

Equipment used to collect samples should be clean and sterile. Sampling tools and sample containers may be purchased pre-sterilized. Alternatively, tools and containers may be sterilized at 121 ^{0}C (250 ^{0}F) for 30 minutes in an autoclave prior to use. Heat-resistant, dry materials may be sterilized in a dry-heat oven at 140 ^{0}C (284 ^{0}F) for 3 hours.

The type of sample containers used will depend on the type of samples collected, but may include pre-sterilized plastic bags, tubes, cups and flasks. Containers should be dry, leak-proof, wide-mouthed, and of a size suitable for the samples. Containers should also seal properly to ensure the sample integrity. Sample containers should be properly labelled prior to starting sample collection.

Sample collectors should wear a clean lab coat, sterile gloves, and a hair net to ensure they do not contaminate the samples. Hands should be washed immediately before sampling, and prior to putting on sterile gloves. Sterile gloves should be put on in a manner that does not contaminate the outside of the glove. Gloves should be properly discarded after use.

Hands should be kept away from mouth, nose, eyes, and face while collecting samples.

Sampling tools should be protected from contamination at all times before and during use. Sampling tools and samples moving between the sampling site and the sample container should not be passed over the remaining pre-sterilized tools.

The sterile sample container should be opened only sufficiently to admit the sample, the sample should be placed directly in the container, and then the container should immediately be closed and sealed. If collecting samples in a container with a lid, the lid and container should be held in one hand while collecting the sample. The lid should NOT be completely removed. (The lid should not be held separately or placed on a counter).

The sample container should be filled no more than 3/4 full to prevent overflow. The air from the container should not be expelled when sealing, particularly for plastic bags. Samples or sampling equipment should not be exposed to unfiltered air currents.

Samples should be delivered to the laboratory and analysed promptly. Perishable material should be kept at an appropriate temperature, preferably at 0 to 4 ^{0}C (32 to 40 ^{0}F). Sealed coolant packs should be used to avoid contamination from melting ice.

Sampling Plan

Sprouters should have a sampling plan in place to ensure the consistent collection of samples in an appropriate manner. They should test for pathogens by collecting a representative sample of

spent irrigation water from each production lot or batch.

For purposes of this guidance, a sprout lot is defined as a quantity of sprouts produced and handled under uniform conditions with as little variation as possible and harvested on the same day (e.g., sprouts produced from a single seed lot, germinated, grown and harvested at the time using the same disinfection and growing methods and type of equipment).

Pooling samples from different sprout lots will allow reduction of the analytical workload. If a presumptive positive is found, the sprouter should discard all lots represented by the pooled sample or perform additional tests to determine which lot(s) is (are) contaminated.

1. Sample Collection for Spent Irrigation Water

The volumes given below for spent irrigation water (or sprouts) represent a sufficient sample size to test for both *Salmonella* spp. and *Escherichia coli* O157:H7.

If testing spent irrigation water, 1 litre of water should be aseptically collected as the water leaves a drum or tray during the irrigation cycle.

If sprouts are grown in drums, a single 1 litre sample may be collected.

If sprouts are grown in trays, and all trays in a production lot have a common trough for collecting spent irrigation water, a 1 litre sample may be collected at that point. If there is no common collection point for water from trays, it may be necessary to collect water samples from individual trays and pool these samples. If the tray is large, it may be necessary to take a sample of water from different areas of the tray. A sampling plan should be devised to ensure collection of a sample that is representative of the production lot. When 10 or fewer trays make up a production lot, approximately equal volumes of water should be collected from each of the 10 trays to make a total sample volume of 1 litre. For example, collect about 100 ml of water from each of 10 trays to make a 1 litre sample: about 125 ml from each of 8 trays, and so on. When more than 10 trays make up a production lot, ten samples should be collected throughout the entire production lot (e.g., if there are 20 trays in a production lot, collect samples from every other tray in the rack moving from top to bottom, side to side, and front to back). Samples should be placed directly in clean, sterile, pre-labelled containers.

2. Sample Collection for Sprouts

If testing sprouts, 5 sample units of approximately 200 grams each should be aseptically collected from different locations in the drum or growing trays. Sample units should be collected throughout the entire production lot (e.g., from top to bottom, side to side, and front to

back of the drum or trays). Each 200 gram sample unit should be placed directly into individual clean, sterile, pre-labelled containers.

Microbial Testing Procedures

The testing procedures described in this guidance were chosen to obtain results as simply and quickly as possible on the presence or absence of two major pathogenic bacteria, i.e., *Salmonella* spp. and *Escherichia* O157:H7. Methods are described in the Health Canada (HC) Compendium of Analytical methods[2].

In addition, seasonal or regional differences in water quality, type of seed being sprouted, individual sprout production factors, and variations in sampling and analytical conditions may all impact the effectiveness of the screening tests.

Test Kits:

Escherichia coli O157:H7:

1. MFLP-87 VIP EHEC. Biocontrol Systems, Inc., Bellview, WA.
2. MFLP-94/95 Reveal *E.coli* O157:H7, Neogen Corp., Lansing, MI.
3. MFLP-91 Tecra UVA method for *E.coli* O157:H7.
4. Any other methods listed in the Compendium for *E. coli* O157:H7.

Salmonella spp.:

1. MFHPB-24 Vidas SLM method, Biomerieux, Montreal.
2. MFLP-96 Reveal kit for *Salmonella.*
3. MFLP-97 Alert kit for *Salmonella.*
4. MFLP-35 Tecra VIA for *Salmonella.*
5. Any other methods listed in the Compendium for *Salmonella*.

[2] http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbio/index_e.html

General Laboratory Instructions

Follow instructions in each method.

Dividing Samples into Sample Units for Analysis

Spent Irrigation Water

A total of 1 L of spent irrigation water should be collected for analysis. Two (2) 100 ml sample units should be analysed for the presence of *E. coli* O157:H7. Two (2) 375 ml sample units should be analysed for the presence of *Salmonella* spp. Any unused portion of spent irrigation water should be stored under refrigeration pending completion of the analysis.

Sprouts

Five (5) samples units of 200 g each should be collected for analysis. For each sample unit, one 25 g sample unit should be analysed for the presence of *E. coli* O157:H7 and one 25 g sample unit should be analysed for the presence of *Salmonella*. Unused portions of the sprout sample units should be stored under refrigeration pending completion of the analysis.