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Appendix E - UNIVERSITY HONORS PROGRAM
SENIOR PROJECT - APPROVAL

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PROJECT TITLE: A Comparison of methods for the
Recovery of *Salmonella* on Cantaloupe
Rinds

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

Signed: David Golden Faculty Mentor

Date: 4/28/2004

General Assessment - please provide a short paragraph that highlights the most significant features of the project.

Comments (Optional):

Comparison of Methods for the Recovery of *Salmonella* from Cantaloupe Rinds

**A Thesis Presented to Satisfy Graduation Requirements for
The University Honors Program
The University of Tennessee, Knoxville**

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Abstract

Recovery of *Salmonella* from cantaloupe using hand massaging, excision, and the Microbial-Vac™ (M-Vac) was compared. Cantaloupe rinds (100 cm²) were inoculated with a five strain mixture of *Salmonella* (6.9 or 2.9 log CFU/100 cm²) and held at 4°C for 24 h. Rind sections were placed in stomacher bags with 100 ml of 0.1% peptone water + 2% Tween 80 and vigorously rubbed through the plastic for two min (hand massaging), pummeled with a stomacher (excision), or vacuum-sampled using the M-Vac system. For the M-Vac, a wet-vacuum sampling device, sterile surface rinse solution (SRS, 100 ml) was applied under low pressure followed by vacuum collection per manufacture guidelines. High and low inoculum rinsates from the three methods, except the low-inoculum M-Vac method, were surface plated (spiral plater) onto XLT4 agar without tergitol (mXLT4) and tryptic soy agar with 50 ppm naldixic acid (TSAN.) For the low inoculum M-Vac method, SRS was vacuum concentrated onto a membrane filter, which was aseptically placed onto mXLT4 agar. Media were incubated for 24 h at 37°C, and typical *Salmonella* colonies were enumerated. For the high inoculum, recovery using the M-Vac and excision was similar (5.5 and 4.9 log CFU/100 cm², $P < 0.05$) while excision and massage (4.3 log CFU) were similar, and M-Vac recovery was better than that of massage. For the low inoculum, the M-Vac recovered 1.2 log CFU/100 cm², while *Salmonella* was not recovered using hand massaging and excision. The M-Vac is a non-destructive and effective method for recovery of *Salmonella* from cantaloupe surfaces. Because of the integrated vacuum concentration option, the M-Vac is superior to traditional hand massaging and excision methods for simple recovery of low numbers of *Salmonella*.

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Introduction

The microbiological safety of fruits and vegetables is a major concern for U.S. regulatory agencies, the produce industry, and consumers. Fruits and vegetables are frequently in contact with soil, animals, and humans during growing, harvesting, and processing. As such, they readily become contaminated with natural microbial contaminants. Produce, such as cantaloupes, that grow in direct contact with the soil are particularly vulnerable to contamination. Soil is a common reservoir of several human pathogens, such as *Salmonella* and *E. coli* O157:H7. Observance of poor agricultural practices, such as use of animal manure for fertilizing crops and irrigating crops with sewage effluents, contributes to an increased likelihood that produce may become contaminated with pathogens. Additionally, it has been shown that melon processing and washing can contribute to the inoculation of the rinds if the wash tank is not cleaned regularly and properly (1). At the consumer level, *Salmonella* has been shown to be able to survive for extended periods of time under refrigeration and is capable of rapid growth on cantaloupe flesh (8, 9).

Pathogenic organisms have been isolated from a wide variety of fruits and vegetables, both imported and domestic (3). Several outbreaks of foodborne illness have resulted from consumption of contaminated watermelon and cantaloupe (4,5,10,13). Cantaloupe, in particular, has been implicated as the vehicle of infection in several outbreaks of salmonellosis. From 1990 to 2001, more than 750 confirmed cases of *Salmonella* infection have been associated with consumption of contaminated cantaloupe (6). Many of these cases comprised multi-state outbreaks, with as many as 30 different states involved. In 1999 and 2000, the U.S. Food and Drug Administration conducted a

large-scale survey of imported and domestic produce to determine the incidence of contamination with foodborne pathogens. Cantaloupe was identified as one of the high-risk produce items, with *Salmonella* being selected as one of three pathogens targeted for investigation (2). This study revealed that as much as 7.3% and 3.1% of imported and domestic cantaloupe, respectively, was contaminated with targeted pathogens.

The research focus in this area has been primarily directed at methods to sanitize product, therefore little attention has been directed towards evaluation of methods for recovery of pathogen populations on cantaloupes. The simplicity of using swabs remains preferable and applicable under most situations. While sponge sampling and excision have been shown to be effective methods for determining microbial populations on beef carcasses, cantaloupe sampling protocols have primarily utilized “hand-washing” of intact fruit inside plastic bags containing relatively large volumes of wash fluid. However, the convoluted surface of webbed cantaloupes renders it difficult to effectively remove organisms that are firmly attached to the surface. Enumeration of low numbers of recovered cells is poor or not possible because of the dilution effect in the large volume of wash fluid. Additionally, the physical size of intact fruits makes handling a washing a cumbersome task. Crevices, convoluted surfaces, and physical damage to the surface of produce create areas that “shelter” microorganisms from sanitizer treatments, as well as impeding removal of the organisms for evaluation.

Compared to sponge methods, excision has been shown to produce better microbial recovery from meat carcasses. Further, this method is generally considered to be the “gold standard of comparison” for other surface microbial collection methods applied to meat or similar food surfaces (6,14). The need for more efficient, repeatable, and non-

destructive food surface contamination sampling is not new. Some recently developed methods include use of rinses and vacuum collection, while others use mechanically assisted sponge scrubbing or adhesion materials applied to the sampling surface. Most have been of limited success. The Microbial-Vac™/M-Vac™ (MV), is a new sampling device that combines multiple techniques to improve food or other-surface sampling efficiencies. The MV (Figures 1 and 2) operates with a patented airflow design and filter system that utilizes Liquid or Air Assisted Microbial Detection and Capture, (LAMDAC™) principles to collect and concentrate surface bacteria. The MV collection protocol incorporates spraying and retrieval of a surface rinse solution (SRS) over a defined surface area. SRS temperature, pressure, and flow rate are controlled during sampling. Detached microorganisms are vacuumed from the surface and collected in SRS, which can then be analyzed in a variety of way. An alternative option is to filter the SRS through an internal 0.45 µm membrane filter to concentrate collected recovered cells, which improves the probability of detecting very low numbers of pathogens in diluted aliquots of captured liquid. This 47 mm diameter membrane filter can also be readily removed for direct culture or other, more rapid lab analyses. The objective of this study was to evaluate the adequacy of three sampling methods for their ability to detect high and low populations of *Salmonella* spp. on the rinds of cantaloupes.

Materials and Methods

Test Strains and Preparation of Inoculum

Nalidixic acid-resistant *Salmonella* Agona (alfalfa-associated outbreak), *Salmonella* Baildon (lettuce/tomato-associated outbreak), *Salmonella* Gaminara (orange juice-associated outbreak), *Salmonella* Michigan (cantaloupe-associated outbreak) and *Salmonella* Montevideo (tomato-associated outbreak) were used in this study. Each serotype was cultured separately in tryptic soy broth containing 50 ppm nalidixic acid (TSBN) at 37°C and transferred at 24 hour intervals. Broth cultures were combined to obtain a mixed culture containing equal proportions of each serotype.

Inoculation of cantaloupes

Cantaloupes were obtained from a local produce supplier and stored at 4°C until used. Three 100-cm² areas were marked on the rind of cantaloupes using a permanent marking pen, were cut from the cantaloupe, and all mesocarp from the sections was removed. The rind sections were then placed in sterile petri dishes. Each rind section was inoculated with 100 µL of the mixed *Salmonella* suspension by placing 10 µL aliquots over the 100-cm² area to yield an initial inoculum population of 6.9 (high-inoculum) or 2.9 (low-inoculum) log CFU per 10 cm². Cantaloupes were then held in a Class 2 biological cabinet for one hour to allow the inoculum to dry, and cantaloupes were then stored for 24 h at 4°C prior to sampling.

Sampling

Massage sampling. After inoculation and storage, the 100-cm² inoculated rind sections of cantaloupes were placed into individual stomacher bags containing 100 mL of

0.1% peptone water (PW) and 2% Tween 80 and massaged by hand for two minutes. An aliquot of the rinse solution was then removed and plated directly.

Excision sampling. After inoculation and storage, the 100-cm² inoculated rind sections of cantaloupes were placed into individual stomacher bags containing 100 mL of 0.1% peptone water (PW) and 2% Tween 80 and pummeled with a stomacher for 2 min at 230 rpm, rest one minute, and pummeled again for 2 minutes. An aliquot of the rinse solution was removed and plated directly.

Wet-vacuum sampling. One hundred mL of Surface Rinse Solution (SRS) 0.1 M phosphate buffer containing 0.05% Triton X 100 was applied as the rinse fluid to each rind section using the Microbial-VacTM/M-VacTM (MV) unit. Rinse fluid was collected directly using the MV (74.5 kPa Hg & 42 liter/min airflow). For the high inoculum, *Salmonella* populations were determined by directly plating the rinse fluid. For the low inoculum level, the rinse fluid was filtered through a 0.45 µm filter to collect recovered cells.

Plating and Enumeration

The rinse solutions from the high inoculum samples and the low inoculum samples from massage and excision sampling and the high inoculum samples from wet-vac sampling were directly spiral plated in duplicate using a Whitley Automatic Spiral Plater (WASP). Samples were plated on XLT4 agar without tergitol (mXLT4) and tryptic soy agar with 50 ppm naldixic acid (TSAN.) Plates were incubated for 24 hours at 37°C. Plates were enumerated using a Protocol automatic plate counter. Presumptive positive colonies from TSAN were further tested for conformation by streaking five

presumptive colonies from each TSAN plate onto mXLT4 agar and incubated for 24 hours at 37°C.

For the low inoculum wet-vac sampling samples, the 45 µm filter was removed aseptically and placed onto the surface of a mXLT4 plate. The plates were incubated for 24 hours at 37°C and were enumerated following this period.

Statistical Analysis

All experiments were performed in triplicate. The log₁₀ counts that were recovered from the cantaloupe rinds using the three methods were averaged and analyzed using the mixed procedure (PROC MIXED) of SAS (Version 8.1; SAS Institute, Cary, NC). A randomized block design with nested treatment arrangement, repeated measures with sampling, and blocking on replication was employed. Means were separated using LSMEANS and significant differences defined at $P < 0.05$.

Results and Discussion

As shown in table 1 and figure 3, the MV system provided comparable recovery to the excision method and better recovery than the massage method with the high-inoculum samples. It should be noted, however, that a possible pre-sampling reduction in cell counts occurred due to cell death that occurred during the overnight storage at 4°C, a reduction that was observed by other researchers working with cantaloupes (13,14.) However, this overnight storage step was performed on all replications, so any losses would have occurred to all samples. With the low-inoculum samples (table 1, figure 4), the MV system gave better recovery than both the excision and massage methods. Because of the M-Vac's integrated vacuum concentration option, it is possible to concentrate and plate the rinse solution from a sample with no need to procure additional equipment. It is for this reason that the M-Vac is superior to the other two methods of sampling in recovering low numbers of cells.

While the M-Vac is best at recovering low numbers of cells from cantaloupe rinds, it does require that a laboratory or company purchase the system. Currently, the M-Vac can be purchased for approximately \$XX. A stomacher system is less expensive than an M-Vac, but the cheapest sampling method by far is the massage method that requires little more than a plastic bag and the appropriate rinse solution. The massage system is also very portable. It requires no electricity and could potentially used to sample cantaloupes in the field whereas the other two systems are large, heavy, and depend on electricity for sampling. The Microbial-Vac can be made portable by use of a cart, but still requires a source of electricity for use.

While all three sampling methods are easy to perform, massage and excision sampling are the easiest of the three methods and would require the least personnel training. The MV system is slightly more difficult to use and would require more initial training. However, once the sampling methods are mastered, they are equally easy to perform and can be performed in roughly the same amount of time. The massage method requires the most labor of the three methods because of the need to physically rub the melon through plastic. Sampling large numbers of melons in this way could lead to fatigue in the hands of the worker while the other two methods require less work.

For this study, each sampling method was tested using a 100-cm² section of cantaloupe rind. This was performed for each method as a source of control, but the massage and M-Vac methods do not require an excision from the rind. These methods can be used to non-destructively sample whole melons, while the excision method does require a section to be removed from the rind for sampling. While whole-melon sampling using the massage method can be awkward because of the size of the melon, M-Vac sampling of whole melons is considerably less awkward and does not require the handling of large bags of melons and wash fluid.

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Tables and Figures

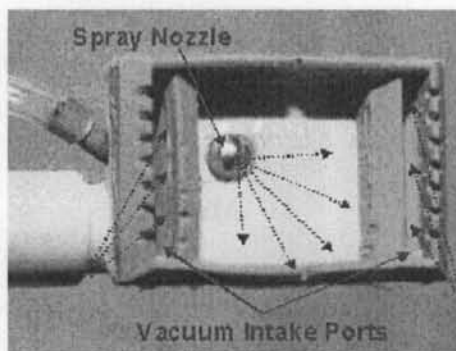


Figure 1. Microbial-Vac™ sampling head.

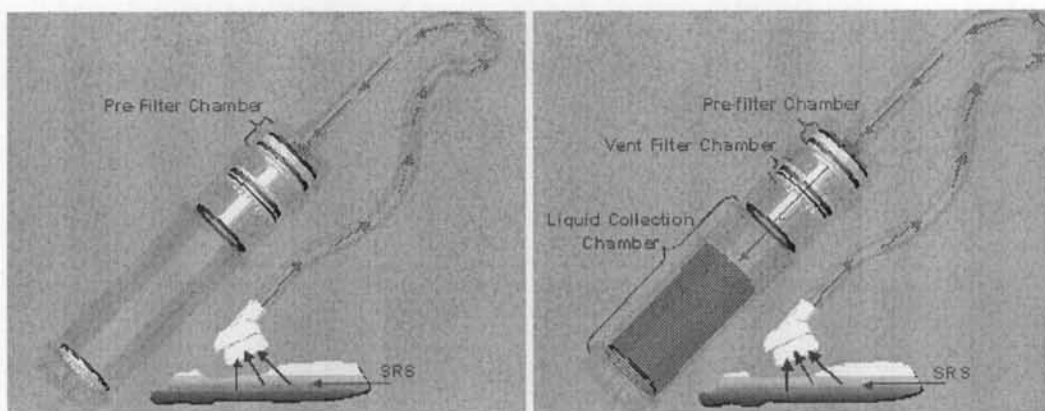


Figure 2. Microbial-Vac™ unit before (left) and after (right) sample collection.

Table 1 - Comparison of recovery^a of *Salmonella* from cantaloupe rinds using three methods of recovery (P<0.05) (N=12)

High Inoculum (6.9 CFU/mL) Samples

Treatment	Mean	Letter Group
Excision	4.8688	AB
Massage	4.3108	B
MV	5.4583	A

Low Inoculum (2.9 CFU/mL) Samples

Excision	ND ^b	B
Massage	ND	B
MV	1.1719	A

a. Recovery measured in log CFU/mL

b. ND = Not Detected

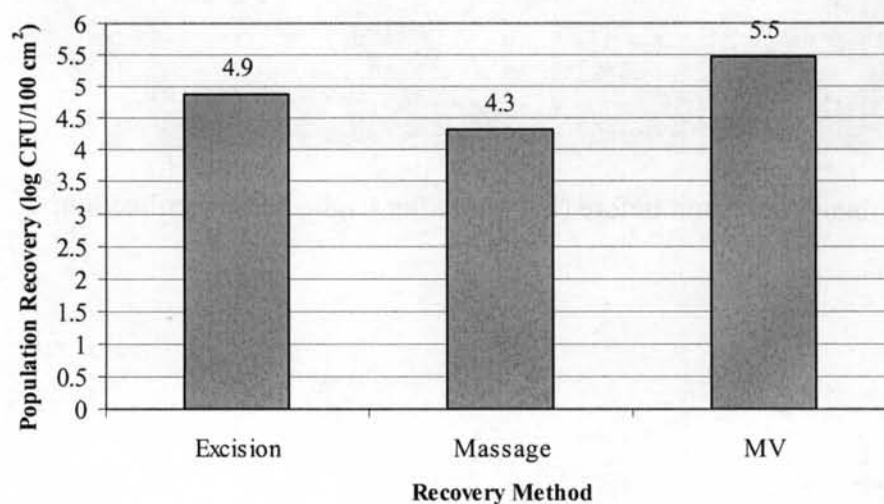


Figure 3 – Comparison of recovery methods (log CFU/100 cm²) of *Salmonella* from cantaloupe rinds; initial inoculum ~log7.0 CFU/100cm² (N=12).

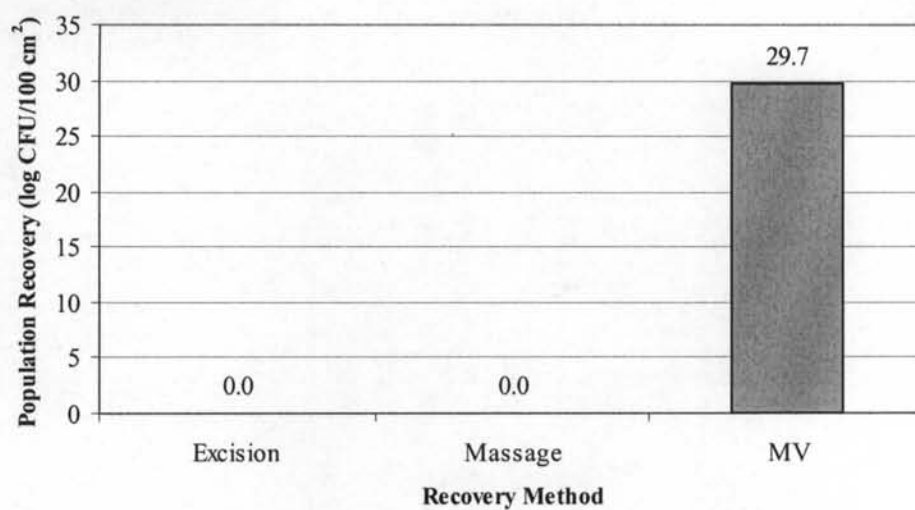


Figure 4 – Comparison of recovery methods (log CFU/100 cm²) of *Salmonella* from cantaloupe rinds; initial inoculum ~log3.0 CFU/100cm² (N=12).