



Reduction of *Salmonella* in Almond hulling and shelling Dusts following application of different sanitizers

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Introduction

In 2000-2001, an outbreak of *Salmonella* Enteritidis phage type 30 (PT 30) (Issac et al. 2005) was linked to the consumption of raw almonds in Canada and the United States. In 2004, CDC reported a second outbreak from raw almonds consumption caused by *Salmonella* Enteritidis (PT9c) (CDC report, 2004). In 2005-2006, a cluster of 15 cases of *Salmonella* Enteritidis NST 3+ was reported suspected to be caused by almond consumption (Ledet et al., 2007).

The function of an almond huller/sheller is to remove the hull and shell of the almond from the nut, or kernel. Orchard debris, soil, and pebbles represent 10 to 25 percent of the field weight of material brought to the almond processing facility. Clean almond kernels are obtained as about 20 percent of the field weight.



Almond "Dust" is generated when hulls and shells are removed from almond kernels. This fine particulate matter is extremely difficult to eliminate from huller sheller (HS) environment (Du et al., 2009). Almond kernels are mixed with hulls, shells and large volumes of dust during processing.

HS facilities have typically cleaned and sanitized by high pressure air-blowing followed by spraying kernel contact surfaces (where exposed) with aqueous QUATs. All dust was not eliminated from surfaces prior to QUAT application. Previous studies (Uesugi and Harris, 2006) have demonstrated *Salmonella* growth in almond hulls and shells when water is added. Dust may an important vector for almond contamination in HS facilities (Uesugi et al. 2007).



Objectives

The objective of this study was to evaluate the efficacy of aqueous and alcohol-based quaternary ammonium sanitizers (QUATs) for reducing *Salmonella* in dusts generated in almond HS facilities.

Materials and Methods

Cultures used

<i>Salmonella</i> Serovars	
Strain	Source
Enteritidis PT 30	Almond outbreak (Issacs et al., 2005)
Enteritidis PT 9C	Almond outbreak (CDC report, 2004)
Montevideo	Almond survey (Danyluk et al. 2007)
Newport	Tomato outbreak (Greene et al. 2008)
Typhimurium	Almond survey (Danyluk et al. 2007)

Sanitizers used

Sanitizers Used	Concentration
Aqueous based QUAT	200 ppm, 58.6% isopropyl alcohol
Alcohol based QUAT	200 ppm

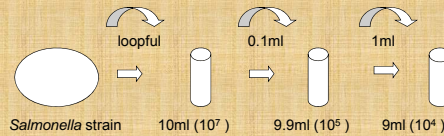
Material and Methods

Preparation of Inoculum

Nalidixic acid resistant *Salmonella* strains were streaked for isolation on tryptic soy agar (TSA) supplemented with Nalidixic acid (50µg/ml) and incubate at 37±2°C for 24±2 hours.

A single colony of each strain was selected from TSA plates with an inoculating loop and suspend in 10 ml sterile deionized water. This generates ca.10⁷ CFU/ml stock inoculums.

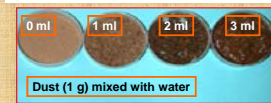
From this stock culture, 0.1 ml was transferred to 9.9 ml sterile water (10⁵ CFU/ml) and 1ml was subsequently transferred into 9ml sterile water (10⁴ CFU/ml). This diluted inoculum was used to inoculate samples of dust.



Use of Sanitizers in Dust



To 50 ml Falcon tubes: 1 g dust, 1 ml inoculum and 2 ml sterile deionized water, aqueous based QUAT or alcohol based QUAT was added.



Tubes of almond dust and *Salmonella* were held at 30±2°C and enumerated at 0 and 48 h.

The samples were run in duplicate and each experiment was replicated three times.

Enumeration

Butterfield's Phosphate buffer (16 ml) was added tubes to achieve a total volume of 20 ml, and vortexed.



Dilutions were plated onto selective media (Bismuth Sulphite agar; BSA supplemented with nalidixic acid (50µg/ml)) in duplicate.

Incubate at 37±2°C for 24±2 hours.



Typical *Salmonella* colonies were counted by hand.

Results were calculated in log CFU/g dust.

Results

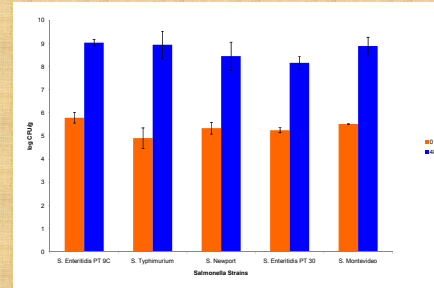


Figure 1: Fate of *Salmonella* strains in almond dust wetted with 2 ml sterile water at 0 hours and 48 hours, plated on BSAN in duplicate (n=6).

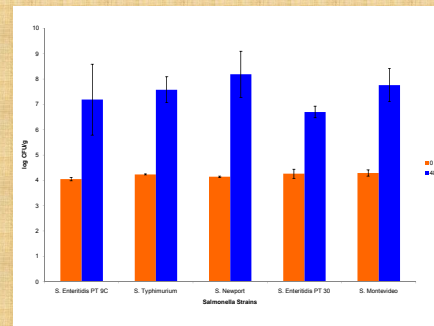


Figure 2: Fate of *Salmonella* strains in almond dust wetted with 2 ml of aqueous based QUAT at 0 hours and 48 hours, plated on BSAN in duplicate (n=6).

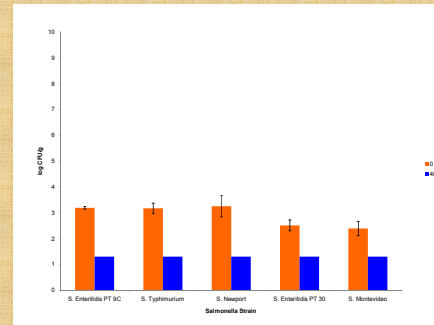


Figure 3: Fate of *Salmonella* strains in almond dust wetted with 2 ml sterile water at 0 hours and 48 hours, plated on BSAN in duplicate (n=6).

Results

• Addition of water to almond dust inoculated with five different strains of *Salmonella*, lead to population increases of ca. 3 log CFU/g after 48 h incubation at 30°C (Figure 1).

• Previous work by Du et al., (2009) demonstrated that similar increases of *Salmonella* Enteritidis PT 30 were seen regardless of initial inoculum levels.

• Addition of 200 ppm aqueous based sanitizer to 1 g dust inoculated with five different strains of *Salmonella* after 48 h incubation at 30°C.

• Previous work Du et al., (2009) demonstrated increasing the concentration from 200 to 1000 ppm of aqueous QUAT did not significantly improve the antimicrobial effect against *Salmonella* Enteritidis PT 30.

• Du et al. (2009) also demonstrated that increasing the volume of aqueous QUAT did not significantly improve the antimicrobial effect against *Salmonella* Enteritidis PT 30.

• Addition of an alcohol based sanitizer to the inoculated dust lead to an immediate reduction of ca. 2 log CFU/g of all five strains of *Salmonella* tested. Following incubation with alcohol QUAT for 48 hours at 30°C reduce the population of *Salmonella* to ≤1.3 log CFU/g.

Summary

• All strains of *Salmonella* tested grew rapidly at 30°C in almond dust wetted with water or aqueous based QUAT.

• Alcohol based QUAT reduced populations of all *Salmonella* to below the detection limit (<1.3 log CFU/g). No *Salmonella* was detected in following 48h of incubation in alcohol based QUAT.

Recommendations

• The use of water or aqueous based QUATs should be restricted in HS facilities where dust cannot be controlled.

• Alcohol based QUAT was effective in reducing *Salmonella* in the presence of high levels of almond dust.

• This should also be considered for other nut production facilities where large volumes of dust may exist.

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