DRY CROSS-CONTAMINATION MODES AND FACTORS ASSOCIATED WITH *SALMONELLA* DURING ALMOND PROCESSING

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Abstract

Recently, there have been numerous outbreaks associated with *Salmonella* in low-moisture foods. According to the CDC, there were multistate outbreaks associated with *Salmonella* for pistachios in 2009 and 2016 and almonds in 2000 and 2003, which caused massive nationwide recalls. This study identifies the environmental and physical factors that affect modes of cross-contamination associated with *Salmonella* in order to enhance a discrete element simulation of almond processing. Almond shell pieces and kernels (200 g) were inoculated with *Salmonella* Enteritidis PT30. Subsequently, the inoculated kernels (5 g), shell pieces (5 g) and un-inoculated kernels (200 g) were conditioned at 0.20, 0.40 and 0.6 a_w, and mixed in a stainless steel drum (140 mm diameter) for a total number of rotations (TNR) of 5, 20, 40, and 80 revolutions. Thereafter, the contaminated samples (5 g) were retrieved, and enumerated for the transferred *Salmonella*. The maximum bacterial load transferred from shells to kernels was significantly higher ($P<0.05$) than that of kernels-to-kernels for 0.2 and 0.6 a_w. When comparing a_w, there was a significant difference between 0.2 and 0.4 ($P<0.05$) for both kernel-to-kernel and shell-to-kernel. This indicates that environmental and physical factors like a_w and surface structure significantly affect the dry transfer of *Salmonella*. Identifying factors affecting bacterial cross-contamination modes is critical information for secondary modeling used in discrete element model simulation, which will reveal the cross-contamination pathways of *Salmonella* for an actual processing system.

Background/Justification

- In 2000 and 2003, there were outbreaks of *Salmonella* in California almonds, resulting in a 13 million pound recall of raw almonds in 2004 (Danyluk 2007).
- There is a lack of understanding of how this contamination occurs and the factors (environmental and physical) that have an effect on the cross-contamination of low moisture products.
- In addition, it is uncertain which areas during almond processing are the highest risk areas for cross contamination (e.g. hulling, shelling, sorting or roasting step).

Objective

To quantify dry cross contamination for *Salmonella* during different stages almond processing (shelling and bulk handling)

References

Danyluk, M. D., Jones, T. M., Abd, S. J., Schlitt-Dittrich, F., Jacobs, M., & Harris, L. J. (2007). Prevalence and amounts of *Salmonella* found on raw California almonds. Journal of Food Protection, 70(4), 820-827.

Methods and Materials

Materials

- The raw, shelled almond kernels treated with propylene oxide (pasteurized) were obtained.
- Raw, in-shell almonds were obtained and broken into pieces. Pieces that passed through a sieve opening of 1 cm^2 were used.

Inoculation

- *Salmonella Enteritidis* PT30 inoculum were added to almond kernels or shells (100 g total).
- Almonds or shell pieces were hand mixed (~2 min) with inoculum in a sterile bag, and dried in a biosafety hood.

Conditioning

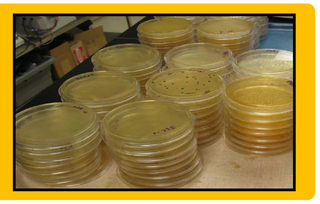
- After drying, the inoculated almonds or shell pieces, and un-inoculated almonds were transferred to a conditioning chamber for water activity (a_w) equilibration.
- Water activity equilibration of the almonds took about 10 days in the conditioning chambers to achieve an target a_w of 0.2, 0.4, and 0.6, respectively.

Cross Contamination Experiment

- The un-inoculated conditioned almonds (200 g) were added to a stainless steel drum (140 mm diameter and 64 mm depth), and combined with 5 g of the inoculated almonds or shells pieces. The drum was rotated at 8, 16, and 24 RPM.
- Four grams samples of almond kernels were extracted from the drum at specific time intervals at 4, 16, 20, and 40 total number of rotation.

Enumeration

- Transferred *Salmonella* were enumerated on Trypticase Soy Agar with Yeast Extract (0.6% w/v) supplemented with ferric ammonium citrate (0.05% w/v) and sodium thiosulfate (0.03% w/v) after 48 hr of incubation at 35°C.



Statistical Analysis

- An equation was used to represent the Log(CFU/g) transferred to clean almond kernels dependent on concentration and distance.
- Statistical difference was determined by fitting the data to the nonlinear equation, and determining if the two parameters, τ (rate constant) and C_a (asymptotic value), were statistically different by a student's t-test ($P<0.05$).

$$\frac{dC}{dt} + \frac{C}{\tau} = \frac{C_a}{\tau}$$

Results

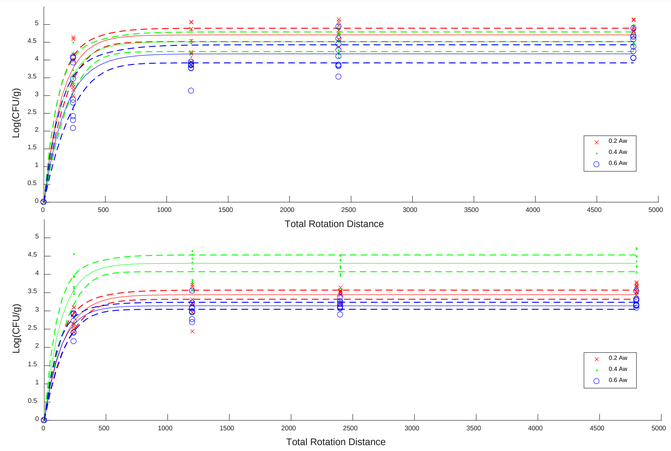


Figure 1: *Salmonella* transferred to clean kernels from contaminated shell pieces (top) and kernels (bottom) at different a_w.

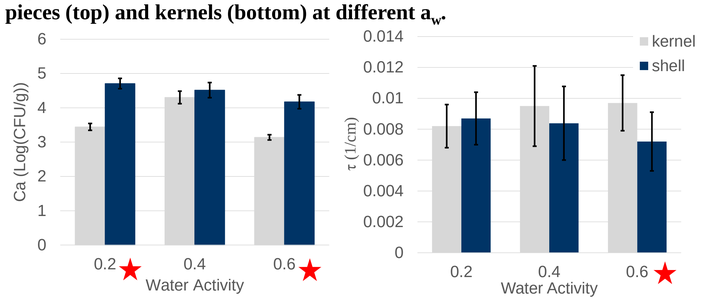


Figure 2: Nonlinear model parameters, C_a (left) and τ (right) of each a_w condition and experiment with 95% confidence intervals.

Conclusion

- Water activity showed a significant effect on cross contamination for *Salmonella* during almond kernel-to-kernel contamination for 0.4 a_w. However, water activity was not a significant factor on transfer using shell pieces.
- There was not a statistical difference in the transfer rate of bacteria when comparing transfer from kernel-to-kernel and shell-to-kernel cross contamination for only 0.2 and 0.4 a_w ($P > 0.05$).
- There was a statistical difference between the asymptotic value reached by kernel-to-kernel and shell-to-kernel cross contamination for 0.2 and 0.6 a_w ($P < 0.05$).
- Overall, water activity and inoculation method are considered as critical to evaluate dry transfer of *Salmonella* during almond processing.

Significance

- Provides secondary modeling to be applied to a discrete element method (DEM) model simulation.

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