Isothermal Inactivation Kinetics of *Salmonella* Montevideo on Partially Dried Apple Cubes

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Abstract

Introduction: The dynamic nature of drying from high to low water activity (a_w) poses challenges in predicting microbial lethality. Additional data on the thermal inactivation kinetics of *Salmonella* on apple cubes can assist in predicting microbial inactivation during drying.
Purpose: To investigate *Salmonella* inactivation on partially dried apple cubes with different a_w during isothermal treatment at various temperatures.
Methods: Gala apple cubes (6.40mm) were pre-dried to a_w 0.45, 0.60 or 0.75. *Salmonella* Montevideo was harvested from lawn culture grown on tryptic soy agar with yeast extract (TSAYE) and inoculated onto the pre-dried apple cubes (3% v/w) to achieve ~8 log CFU/g population. After ~96h re-equilibration (45, 60, or 75% RH), inoculated apple cubes were packed into aluminum test cells under controlled RH and isothermally treated in a water bath. At various time points (n=6), triplicate samples were collected and cooled in an ice-water bath, and *Salmonella* was enumerated on TSAYE with ammonium iron citrate and sodium thiosulfate.
Results: Following post-inoculation equilibration at 45, 60, and 75% RH, the a_w of apple cubes was 0.44 ± 0.01, 0.61 ± 0.02, and 0.76 ± 0.01, respectively. *Salmonella* populations post-equilibration were significantly greater ($p<0.05$) on apple cubes at a_w 0.45 and 0.60 (8.46 ± 0.20 and 8.48 ± 0.23 log CFU/g, respectively) than 0.75 (7.89 ± 0.27 log CFU/g). At a_w 0.45, D-values were 12.93 ± 0.33, 4.70 ± 0.10, and 1.71 ± 0.07 min at 67.5, 75.0, and 87.5.°C, respectively. At a_w 0.60, D-values were 35.92 ± 1.36, 10.50 ± 0.26, and 3.07 ± 0.14 min at 60.0, 67.5, and 75.0°C, respectively. At a_w 0.75, D-values were 41.82 ± 0.39, 9.57 ± 0.36, and 2.19 ± 0.17 min at 52.5, 60.0, and 67.5°C, respectively. Highest and lowest ($p<0.05$) D-values were found on a_w 0.45 and 0.75 apple cubes, respectively. Similarly, the z-value was highest ($p<0.05$) for a_w 0.45 apple cubes (17.06 ± 0.43°C) and lowest for a_w 0.75 apple cubes (11.71 ± 0.38°C).
Significance: Results demonstrate that lower a_w in apple cubes led to higher *Salmonella* thermal resistance (D-value) and a higher z value.

Introduction

In a previous study (1), process parameters such as temperature and humidity were used in combination with D-values in predicting the inactivation of *Enterococcus faecium* during cookie baking. With a similar approach, the isothermal kinetic of *Salmonella* on apple cubes (D and z values) could also assist in predicting microbial inactivation during dynamic drying.

Objective

The objective is to investigate the isothermal kinetics of *Salmonella* on apple cubes as affected by apple water activity during isothermal treatment. Apples with different water activities are to be examined at various temperatures.

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USDA

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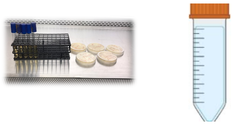
Materials and Methods

1. Apple pre-conditioning

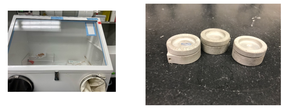
Gala apples were cut into cubes (6.4 mm) and dried with a home-style convection oven to achieve various targeted water activities (0.45, 0.6, and 0.75). Dried apple cubes were stored in humidity-controlled (45, 60, and 75%) chambers until inoculation studies.

2. Apple inoculation and post-inoculation conditioning



Salmonella Montevideo was harvested from plate grown cells (~10-11 log CFU/mL) and mixed with pre-conditioned apple cubes (3% v/w) to achieve ~8 log CFU/g. Inoculated apple cubes were stored in humidity-controlled chambers for 4 days before isothermal treatments.

3. Test cell packing

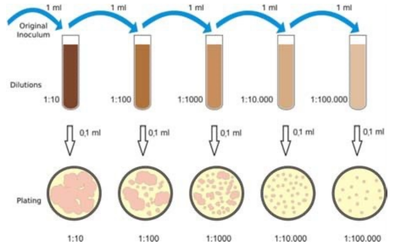


Prior to isothermal treatments, ~10 pieces (~0.8g) of apple cubes were packed into aluminum test cells under controlled relative humidity (45, 60, and 75%). Test cells were tightly packed to avoid creating headspace.

4. Isothermal treatment

Isothermal treatments were performed at 3 temperatures for each apple a_w level (n=3) and 3 independent trials were completed. Test cells (n=18) were submerged in a water bath simultaneously. At set time points (n=6), test cells (n=3) were immediately removed from the water bath and cooled in the ice-water bath. Samples were then aseptically transferred into sample bags.

5. *Salmonella* enumeration



Samples were serially diluted in BPW, plated on TSAYE + Ammonium Ion Citrate + Sodium Thiosulfate, incubated (37°C, 24 h), and then enumerated.

Results

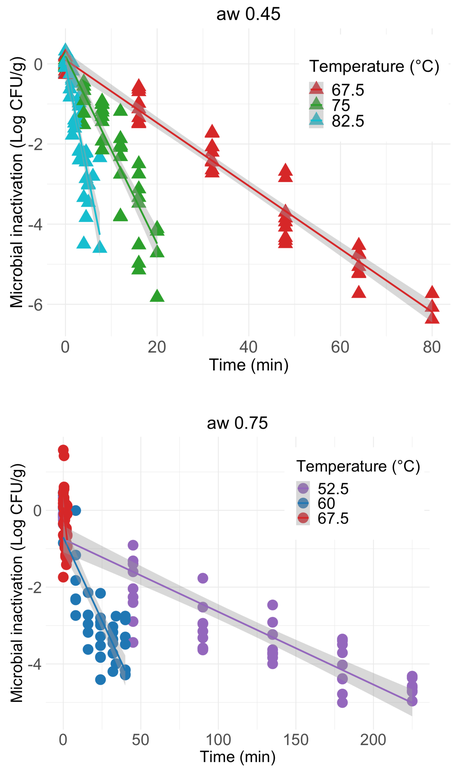


Figure 1. The isothermal inactivation of *Salmonella* in apple cubes with water activity 0.45, 0.60, and 0.75. The colored lines represent linear regression trend lines. The grey bands represent 95% confidence intervals.

Table 1. The D-values of *Salmonella* in apple cubes with a water activity of 0.45, 0.60, and 0.75 and the Z_T values at each water activity level.

a_w	0.45	0.60	0.75
$D_{52.5°C}$			41.82 ± 0.39 min
$D_{60.0°C}$		35.92 ± 1.36 min	9.57 ± 0.36 min
$D_{67.5°C}$	12.93 ± 0.33 min	10.50 ± 0.26 min	2.19 ± 0.17 min
$D_{75.0°C}$	4.70 ± 0.10 min	3.07 ± 0.14 min	
$D_{82.5°C}$	1.71 ± 0.07 min		
z	17.06 ± 0.43°C	14.04 ± 0.38°C	11.71 ± 0.38°C

Discussion

- On 0.45 a_w apple cubes, *Salmonella* D-values ranged from 1.71 to 12.93 min when treated under temperatures ranging from 82.5 to 67.5°C. A significant difference was found in the D-value among all 3 temperatures.
- On 0.60 a_w apple cubes, *Salmonella* D-values ranged from 3.07 to 35.92 min when treated under temperatures ranging from 75.0 to 60.0°C. A significant difference was found in the D-value among all 3 temperatures.
- On 0.75 a_w apple cubes, *Salmonella* D-values ranged from 2.19 to 41.82 min when treated under temperatures ranging from 67.5 to 52.5°C. A significant difference was found in the D-value among all 3 temperatures.
- At 67.5°C, the highest ($p<0.05$) D-value was found at the lowest a_w (0.45), and vice versa.
- A lower z-value ($p<0.05$) was found in apple cubes with higher a_w.

Significance

- When combined with the apple a_w and isothermal treatment temperature, the calculated D-values that represent the *Salmonella* thermal resistance could be used as a parameter in predicting *Salmonella* reduction during dynamic apple drying.
- The negative correlation found between apple a_w and z-values allows secondary modeling in which apple a_w is used to predict z-values which represent the change in the thermal resistance of *Salmonella* based on the change in temperature.

Next steps

- Complete isothermal inactivation study for apple cubes with a_w 0.90.
- Examine the effect of apple a_w on *Salmonella* thermal resistance.
- Perform primary and secondary model selection.

Reference

1. Suehr QJ, Liu X, Grasso-Kelley EM, Anderson NM. Predictive Microbial Modeling of *Enterococcus faecium* NRRL B-2354 Inactivation during Baking of a Multicomponent Low-Moisture Food. *J Food Prot*. 2021 Nov 1;84(11):1990–2001.

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