Recovery of *Salmonella* from Steam and Ethylene Oxide-Treated Spices Using Supplemented Agar with Overlay

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ABSTRACT

Introduction: *Salmonella enterica* has been recovered from a number of low water activity foods including spices. Spices may be treated with steam and/or ethylene oxide to reduce microbial loads. Therefore, improved recovery of viable *Salmonella* in treated spices is needed to ensure processes are properly validated.

Purpose: The efficacy of agar overlay with or without media supplementation was examined to improve recovery of *Salmonella* on peppercorn and cumin seeds treated with steam and ethylene oxide.

Methods: Peppercorns and cumin seeds were inoculated with *Salmonella*, dried to 0.3-0.4 a_w, and treated with steam under vacuum (65.4-69.7°C) for 15 s dwell time. Following steam treatment, spices were stomached for 1 min in 0.1% peptone + 0.1% Tween, serially diluted, and plated onto TSA, XLT4 or TSA with added supplements followed by XLT4 overlay. TSA was supplemented with sodium pyruvate (9.09 mmol) + yeast extract (0.6%) [SPY], 3,3'-thiodipropionic acid (5.6 mmol)[TDP], glycerophosphate (10 mmol)[GP], lactate (10 mmol) [LAC], or mannitol (100 mmol) [MAN]. Plated samples were incubated (37°C) for 3 h then molten XLT4 medium was used to overlay. Plates were then incubated at 37°C for 21 h prior to colony enumeration. For peppercorns and cumin seeds subjected to ethylene oxide treatment, TSA was supplemented with SPY, TDP, GP, ATP (8.34 mmol), guanine (64.5 mmol), and magnesium (52.5 mmol). Samples were plated as described above.

Results: TSA supplemented with SPY or TDP resulted in better recovery of *Salmonella* from steam-treated peppercorns (P<0.05). No supplement was associated with improved recovery of *Salmonella* on cumin seeds (P>0.05) following steam treatment. For ethylene oxide-treated peppercorns and cumin seeds, no supplement improved recovery of *Salmonella* (P>0.05).

Significance: For steam-treated peppercorns, supplementation of TSA with sodium pyruvate + yeast extract or 3,3'-thiodipropionic acid may improve recovery of *Salmonella* when overlay plating methods are used.

Figure 1. XLT4 agar plate with typical black colonies produced by *Salmonella*

Figure 2. Steam apparatus with thermocouples and spice holding wire mesh tray

OBJECTIVES

I. Determine the influence of three plating methods, traditional overlay, thin agar overlay, and solid agar overlay, on recovery of Salmonella on peppercorns subjected to steam treatments.

II. Test the efficacy of media supplemented with 3,3'-thiodipropionic acid, pyruvate + yeast extract, glycerophosphate, mannitol, and lactate to recover Salmonella on peppercorns and cumin seeds subjected to steam treatments.

III. Test the efficacy of media supplemented with 3,3'-thiodipropionic acid, pyruvate + yeast extract, glycerophosphate, guanine, ATP, and Magnesium to recover Salmonella on peppercorns and cumin seeds subjected to ethylene oxide treatments

MATERIALS and METHODS

Day 1: Frozen cultures streaked onto XLT4 plates and incubated at 37°C for 24 hours

Day 2: Single colony transferred from XLT4 to 10ml TSB tubes and incubated at 37°C for 24 hours

Day 3: 1 mL of each Salmonella strain was spread onto 2 large TSA plates incubated at 37°C for 24 hours

Day 4:Cells were suspended with 0.1% BPW and cotton swab. Cells were collected with pipette and dispensed into conical screw cap tube. 20 mL of cocktail was applied to each 50 g batch of spice in a Whirl-pak bag and shaken for 1 minute. Inoculated seeds were spread on covered baking sheets and allowed to dry for 24hrs to dry to 0.3-0.5 A_w

Day 5:Once spices reached desired a_w, 50g of each spice was put into muslin drawstring sachets (4x6 in). Thermocouples were placed inside the sachets. The sachets were placed on a metal mesh tray inside a steam apparatus and treated with steam (150°F) for 15 seconds.

Day 5: Following treatment, 10g of spices were put into a stomacher bag containing 90ml of BPW. The spice was stomached for 1 minute before being transferred to sterile vacuum flasks with filter paper (20-25 micron particle retention). Serial dilutions using BPW were made as appropriate from the filtrate. The traditional overlay was used independently and in combination with supplemented TSA to recover *Salmonella* cells injured by the steam treatment. Plates were incubated for 24 hours at 37°C.

Day 6: Plates were counted and data was recorded following incubation.

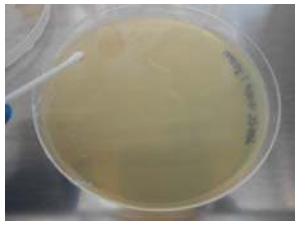


Figure 3. TSA agar plate with lawn of *Salmonella* for wet scrape inoculation

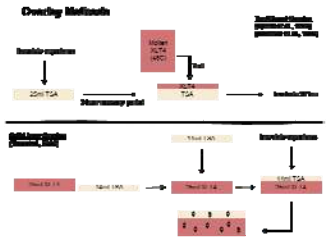


Figure 4. Illustration of Traditional Agar Overlay and Solid Agar Overlay

RESULTS

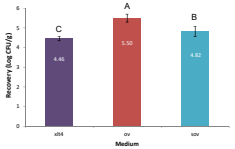
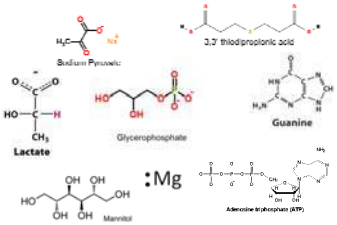


Figure 5. Comparison of overlay methods for recovery of *Salmonella* on peppercorns subjected to a steam treatment. n=4, error bars= standard deviation, letters represent significance level



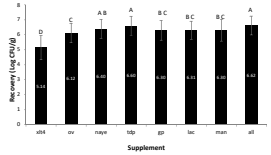


Figure 6. Comparison of supplements on recovery of *Salmonella* on peppercorns subjected to a steam treatment. n=4, error bars= standard deviation, letters represent significance level

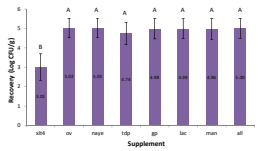


Figure 7. Comparison of supplements on recovery of *Salmonella* on cumin seeds subjected to a steam treatment. n=3, error bars= standard deviation, letters represent significance level

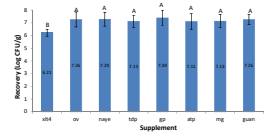


Figure 8. Comparison of supplements on recovery of Salmonella on peppercorns subjected to ethylene oxide fumigation. n=2, error bars= standard deviation, letters represent significance level

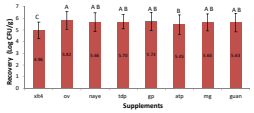


Figure 9. Comparison of supplements on recovery of Salmonella on cumin seeds subjected to ethylene oxide fumigation. n=4, error bars= standard deviation, letters represent significance level

CONCLUSIONS

- XLT4 alone is not a suitable recovery medium for *Salmonella* when validating processes
- Traditional Overlay is the most efficient overlay method to recover *Salmonella*
- Supplementation (TDP) may permit additional repair beyond overlay in certain situations

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