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 aw, and treated with steam under vacuum (65-69.7°C) for 15 s dwell time. Following steam treatment, seeds 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)[GIP], lactate (10 mmol)[LAC], or mannitol (100 mmol)[MAN]. Plates 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.

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 dispersed 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 24 hrs to dry to 0.3-0.5 aw.

Day 5: Once spices reached desired aw, 50g of each spice was put into muslin drawstring sachets (446 mm). 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 6: Plates were counted and data was recorded following incubation.

RESULTS

• 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

CONCLUSIONS

REFERENCES

