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Evaluation of preparation steps used to inoculate almonds with *Enterococcus faecium* NRRL B-2354

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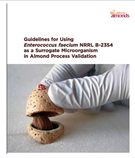
Introduction

Enterococcus faecium NRRL B-2354-inoculated almond kernels, prepared with a standard method, are used to validate reduction of *Salmonella* during thermal treatments.

In this study, the impact of the addition of yeast extract to tryptic soy broth or agar, incubation times, volume for seeding agar lawns, or inoculum volume per 400 g of almonds on levels of *E. faecium* and on inoculated almond drying times were assessed.



Methods

Inoculum preparation of *E. faecium* NRRL-B2354

Tryptic soy agar (TSA) or broth (TSB); TSA or TSB with 0.6% yeast extract (TSAYE or TSBYE, respectively).

Standard Method *

Streak frozen (-80 ºC) culture

DAY 1

Streak onto TSA* (37 ºC for 24 ± 2 h) | Streak onto TSAYE (37 ºC for 24 ± 2 h)

DAY 2

Transfer one isolated typical colony to 10 mL of TSB* (37 ºC for 24 ± 2 h) | Transfer one isolated typical colony to 10 mL of TSBYE (37 ºC for 24 ± 2 h)

DAY 3

Transfer loop of broth culture into 10 mL of TSB* (37 ºC for 18* or 24 ± 2 h) | Transfer loop of broth culture into 10 mL of TSBYE (37 ºC for 18 or 24 ± 2 h)

DAY 4

Enumerate 18 and 24 h TSB culture. Spread culture onto single TSA* plates (150 × 15 mm) 24-h culture: 1 mL*, 0.5 mL, or 0.25 mL/plate; 18-h culture: 1 mL/plate 37 ºC for 24 ± 2 h

Enumerate 18 and 24 h TSBYE culture. Spread culture onto single TSAYE plates (150 × 15 mm) 24-h culture: 1 mL, 0.5 mL, or 0.25 mL/plate; 18-h culture: 1 mL/plate 37 ºC for 24 ± 2 h

DAY 5

Add 6 mL of 0.1% peptone to each TSA or TSAYE plate, loosen bacterial lawn with a sterile spreader, and use a sterile pipette to collect cells into a sterile container.

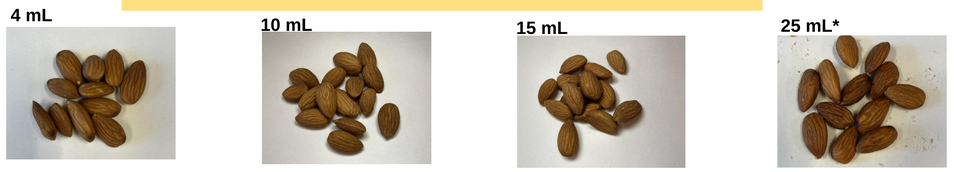
Volume impacts on inoculum distribution on almonds

Raw almond kernels before inoculation:
Water activity: 0.5220 ± 0.022; Moisture content: 5.14 ± 0.13%

4 mL, 10 mL, 15 mL, and 25 mL* of prepared *E. faecium* culture per 400 g of almond kernels (0.5 mL from TSB incubated for 24 h plated onto TSA incubated for 24 h; 15 plates)

Dry the inoculated almonds at ambient temperature and relative humidity to a moisture content of 4.0–5.5%

Measure the population of *E. faecium* in five 10-g samples



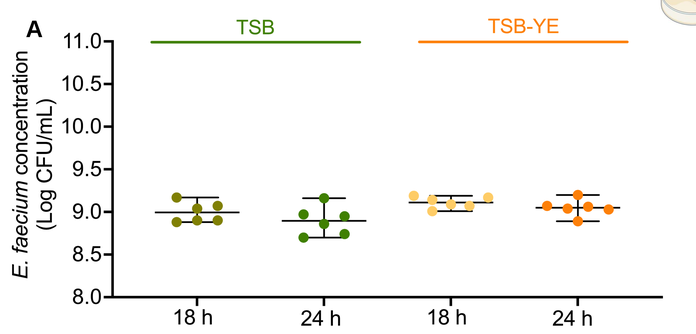
Determine water activity and moisture level of almonds

Homogenize 40 g of almond in food processor for 20 s → Eliminate large particles using #12 sieve and collect the fine ground samples in a tray → Measure moisture content (Moisture Analyzer) and water activity (water activity meter)

Results

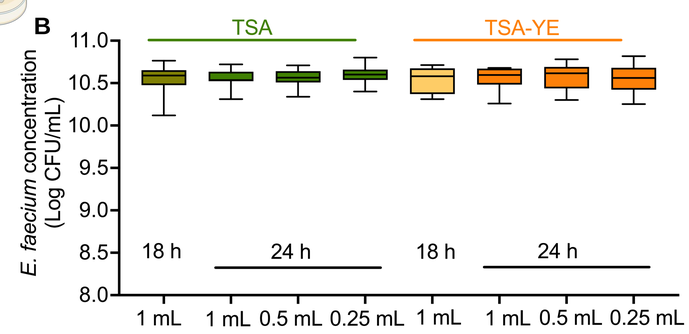
Addition of yeast extract, incubation time, or volume plated did not significantly impact populations of *E. faecium*

Population of *E. faecium* in 2nd culture (TSB or TSB-YE) incubated for 18 or 24 h



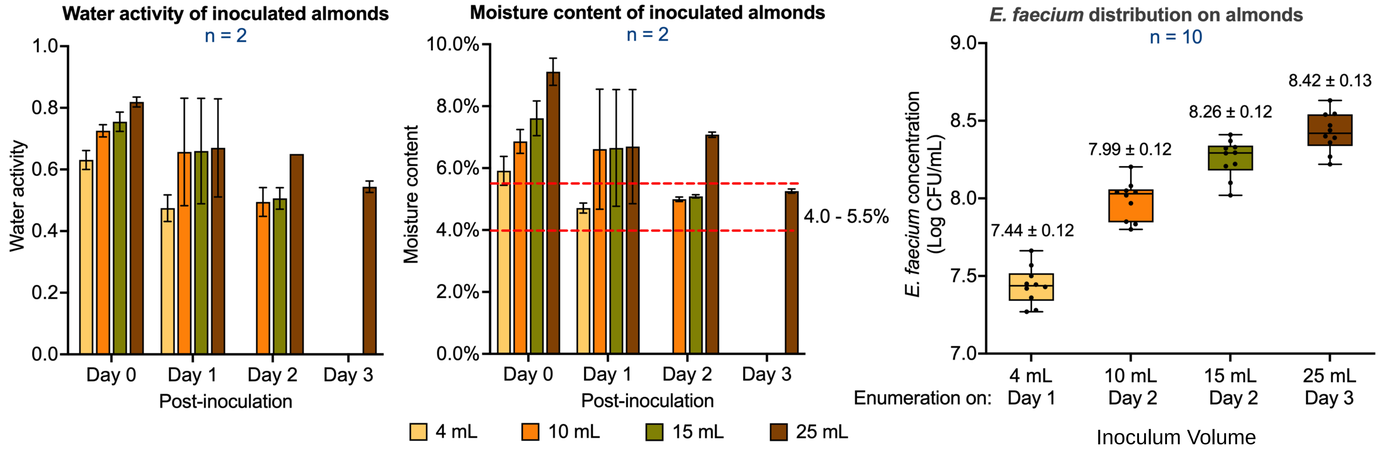
A: n = 6. The maximum and minimum values are denoted by the whiskers; and the median is denoted by the center line.

Population of *E. faecium* in collected bacterial lawn



B: n = 10. For each box plot the maximum and minimum values are denoted by the whiskers; the 75th and 25th percentile are denoted by the top and bottom of the box, respectively; and the median is denoted by the center line.

No significant change in *E. faecium* populations on dried almonds when inoculum was reduced from 25 to 15 mL per 400 g



Ambient temperature and relative humidity was 22.1 ± 0.71 ℃ and 41.1 ± 15.84%, respectively.

Summary

- The addition of yeast extract (0.6%) did not significantly change ($P > 0.05$) recovered populations of *E. faecium* NRRL-B2354.
- No significant difference ($P > 0.05$) in recovered populations of *E. faecium* observed when 0.25, 0.5, or 1 mL was plated onto 150 × 15 mm plates.
- Mean levels of *E. faecium* of ≥8 log CFU/g of almonds were recovered when 400 g of almonds were inoculated at 15 or 25 mL but not at 4 or 10 mL.
- In one of two trials, drying time was reduced by 1 day when inoculum was lowered from 25 to 15 mL per 400 g of almonds.

Acknowledgements

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