Resistance of spice-related *Salmonella* serotypes and *Pediococcus faecium* NRRL B-2354 to dehydration, gamma-irradiation and dry storage

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INTRODUCTION

Validating control measures for pathogen control is paramount in ensuring the effectiveness of food safety programs. Validation is especially needed for low moisture foods, such as spices, since increased pathogen thermal resistance has been reported. Using spice-related strains (SRSS) will increment the accuracy of the results of validation studies, since non-SRSS may not accurately represent the potential adaptation to dry environments (3). For in-plant validation, the use of adequate surrogate bacteria is necessary since pathogens cannot be introduced in food processing environments. Dry-inoculation methods using the same spices or inert powders, such as talcum powder, as a vehicle of *Salmonella,* have been used for challenge studies in low-moisture foods (2). The objective of this research was to test the resistance of SRSS, non-SRSS, and *Pediococcus faecium* to desiccation, irradiation and dry storage.

MATERIALS AND METHODS

1. Inoculation of dry matrices and enumeration of microorganisms

Fifty-gram batches of sterile talcum powder (TP) and onion powder (OP) were inoculated separately with cocktails of *Salmonella* and *Pediococcus faecium* (PF) containing 10^{10} cells/ml.

Spice related *Salmonella* (SRSS)	Non-spice related *Salmonella* (non-SRSS)	*Pediococcus faecium* (PF)
S. enterica ser. Rissen SAL1449	*S. enterica* ser. Choleraeasuis ATCC-13312	*P. faecium NRRL B-2354*
S. enterica ser. Montevideo SAL4599	*S. enterica* ser. Enteritidis ATCC-4931	
	S. enterica ser. Newport ATCC-6962	
	S. enterica ser. Typhimurium ATCC-700720	

The inoculated and homogenized TP batches were dried at 35 °C until reaching 0.45 Aw (1). The OP batches were dried at 25˚C until reaching the original Aw of 0.3. Enumeration of *Salmonella* or *Pediococcus* was performed by diluting one-gram TP or OP samples in 99 ml of peptone water and pummeled for 1 min at 230 rpm. Serial decimal dilutions of the TP or OP homogenate were surface-spread onto TSA or TSA +0.5% K_2SO_3, respectively. Plates were incubated at 35 °C for 3 h and then overlaid with XLT4 or KFS to recover stressed *Salmonella* and *P. faecium* respectively, continuing incubation up to 24 h (4).

2. Effect of desiccation using an inert powder and onion powder

TP, as an inert medium, and OP were used to test the effect of desiccation on the survival of *Salmonella* and *P. faecium* at 35˚C and 25˚C, respectively. The survival of each group of bacteria was measured by inoculating sterile TP or OP, as previously described, and comparing their concentration before and after desiccation.

3. Gamma irradiation treatments

Two-g samples of dried inoculated TP and OP sets were gamma-irradiated at the University of Iowa Radiation Research laboratory with a J.L. Shepard and Associates Model 81 irradiator with a Cs^{137} source and an output of 24.5 Gy/min. D_{10}-values were calculated as the negative inverse of the slope of the line obtained by plotting the microbial populations at the applied irradiation dose, and the doses to achieve a 5 $\log_{10}$ reduction were determined.

4. Survival during storage

The survival of *Salmonella* and *P. faecium* were monitored in TP and OP stored at 25, 4, and -18 °C during 15 weeks.

RESULTS AND DISCUSSION

Effect of desiccation

Non-SRSS strains showed a higher susceptibility to desiccation than SRSS strains and PF in TP and OP (P<0.05), while PF showed the highest resistance (P<0.05).

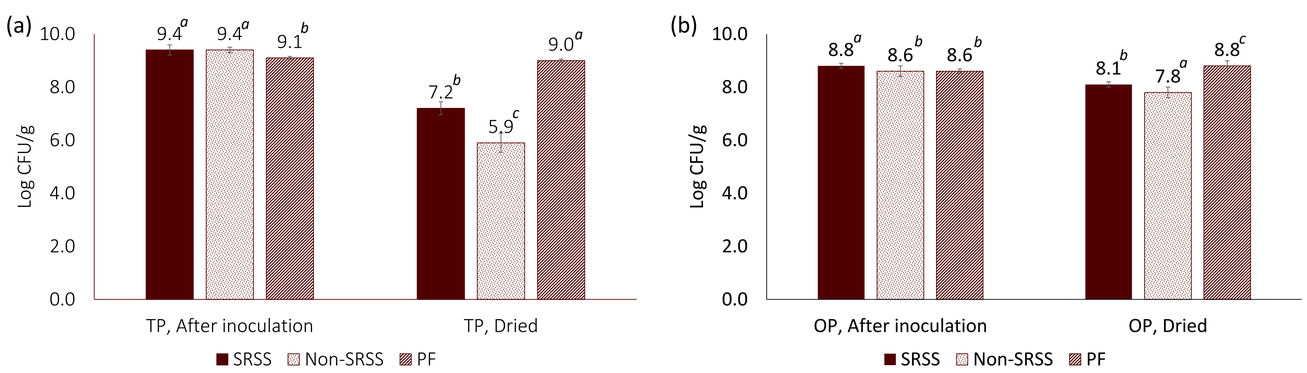

Fig. 1. Resistance to desiccation of *Salmonella* spp. and *P. faecium* in (a) talcum powder and (b) onion powder. Columns represent mean Log_{10} count of each group of microorganisms after inoculation and after desiccation.

[a,b,c]Mean Log_{10} values within the same treatment and same powder, with different superscripts are statistically different (P<0.05).

Gamma irradiation treatments

Table 1. D_{10} values and doses if radiation required to achieve a 5 $\log_{10}$ reduction of *Salmonella* and *Pediococcus* in talcum powder and onion powder

Bacteria inoculated	D_{10} values (kGy)		Dose required to achieve 5 $\log_{10}$ reduction (kGy)	
	Talcum powder	Onion powder	Talcum powder	Onion powder
P. faecium	0.74 ± 0.01[A*]	1.70 ± 0.13[A]	3.70	8.50
SRSS	0.67 ± 0.06[B]	1.01 ± 0.06[B]	3.35	5.05
Non-SRSS	0.62 ± 0.03[B]	0.75 ± 0.14[C]	3.10	3.75

*Mean D_{10} values within a column with different superscripts are statistically different (P<0.05).

Survival during storage

Talcum powder: SRSS, non-SRSS and PF showed no significant differences by the end of the 15th week of storage at -18, 4 and 25 °C (P<0.05). However, the concentration of non-SRSS had more variability than SRSS during the 15-week storage period (Fig 2-3).

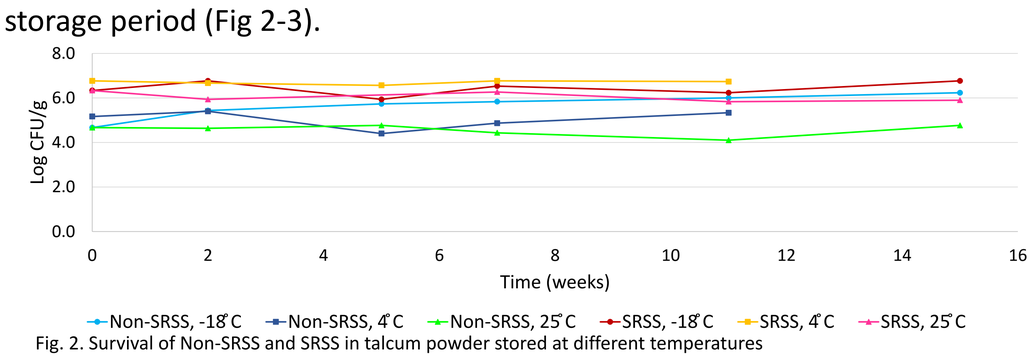

Fig. 2. Survival of Non-SRSS and SRSS in talcum powder stored at different temperatures

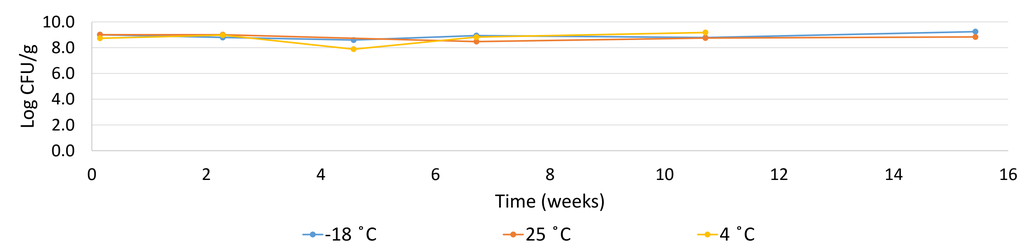

Fig. 3. Survival of *P. faecium* in talcum powder stored at different temperatures

Onion powder: Temperature of storage affected the survival of *Salmonella* spp. and *P. faecium* in OP. In general, the greatest reduction for the 3 groups of microorganisms was at 25˚C with 3.6, 3.6, and 1.9 log cycles for non-SRSS, SRSS, and PF, respectively. SRSS and non-SRSS had similar reduction patterns for all three temperatures and no statistically significant differences were detected (P>0.05) (Fig 4-5).

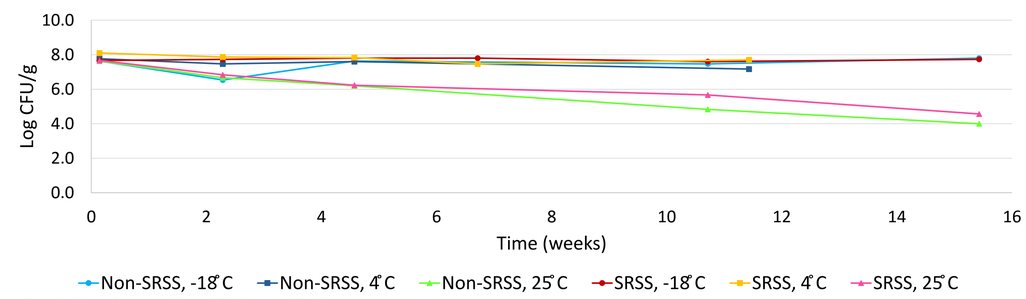

Fig. 4. Survival of Non-SRSS and SRSS in onion powder stored at different temperatures

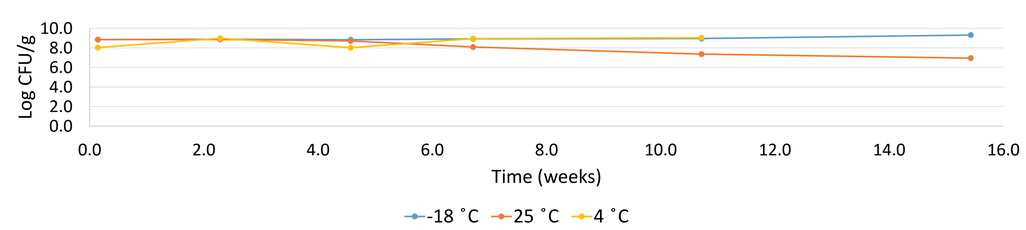

Fig. 5. Survival of *P. faecium* in onion powder stored at different temperatures

CONCLUSIONS

SRSS showed a higher resistance to desiccation than the non-SRSS. The gamma irradiation dose to achieve a 5 log reduction did not differ between SRSS and non-SRSS when using TP, whereas the SRSS cocktail was significantly more resistant to irradiation than non-SRSS in OP. These results emphasize the importance of selecting adequate strains of pathogenic bacteria when designing challenge studies. PF was more resistant than SRSS and non-SRSS to dehydration, irradiation, and storage at different temperatures, supporting its potential use as a surrogate of *Salmonella*.

ACKNOWLEDGMENTS

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REFERENCES

1. Arias-Rios *et al.,* 2015. Standardizing an oregano inoculation procedure for use in challenge studies on reduction of *Salmonella* in dry spices. Poster session 1 presented at the IAFP, Portland, OR. July 26, 2015.
2. Blessington *et al.,* 2013. A dry-inoculation method for nut kernels. *Food Microbiol.* 33:292-297.
3. Hiramatsu *et al.,* 2005. Ability of shiga toxin-producing *Escherichia coli* and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Appl. Environ. Microbiol.* 71:6657-6663.
4. Kang and Fung. 2000. Application of thin agar layer method for recovery of injured *Salmonella* Typhimurium. *J. Food Prot.* 54: 127-132.