Surrogate Organisms for Low Moisture Foods: Tables and References

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Table 1. Studies that compare the survival of surrogate organisms to one or more target pathogens in low moisture foods under different processes

Process type	Treatment	Surrogate organism	Target pathogen(s)	Matrix	Summary	References
Thermal	Extrusion	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Branderup NVSL 96-12528, Oranienburg NSVL 96-12608, Typhimurium ATCC 14028, Enteritidis IV/NVSL 94-13062, Heidelberg/Sheldon 3347-1	Balanced carbohydrate-protein meal	Extrusion led to a 5-log reduction of *Salmonella.* *E. faecium* showed greater heat resistance than *Salmonella.*	Bianchini et al., 2014
	Stagnant and forced dry air heating (peanuts), hot oil (pecan kernels), hot water (in-shell pecans)	*Enterococcus faecium* ATTC 8459[1]; *Enterococcus faecalis* ATTC 29212	*Salmonella* strains: Senftenberg 775W ATCC 43845, Enteritidis PT 30 ATCC BAA-1045, Tennessee K4643	Peanuts, pecans	*E. faecium* survived better or not significantly worse than *Salmonella* in all tested processes.	Brar and Danyluk, 2019
	Heat process	*Pediococcus acidilactici* (from starter culture (Formula 100, Trumark, Linden, N.J.)	*Escherichia coli* O157:H7 strains: ATCC 43894, ATCC 51657, ATCC 51658, ATCC 43895; *Salmonella* strains: Typhimurium S9, Heidelberg S13, Enteritidis E40, Infantis S20, Hadar S21	Beef jerky	*P. acidilactici* displayed greater thermal resistance than all pathogens evaluated when reductions could be calculated.	Buege et al., 2006

Process type	Treatment	Surrogate organism	Target pathogen(s)	Matrix	Summary	References
	Water blanching, steam blanching	*Pediococcus acidilactici* ATCC 8042; *Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Anatum, Montevideo, Senftenberg 775W, Tennessee, Schwarzengrund, Infantis, Mbandaka	Pet food	Both *P. acidilactici* and *E. faecium* showed greater thermal tolerance than the *Salmonella* cocktail.	Ceylan et al., 2015
	Heat process	*Enterococcus faecium* ATTC 8459; *Saccharomyces cerevisiae*	*Salmonella* strains: Typhimurium, Newport, Senftenberg 775W	Wheat flour	*E. faecium* was more heat resistant than the *Salmonella. S. cerevisiae* was less heat resistant than *Salmonella*.	Channaiah et al., 2016
	Heat process	*Escherichia coli* K12 LMM 1010, *Escherichia coli* P1 ATCC BAA-1427; *Listeria innocua* ATCC 33090; *Enterococcus faecium* NRRL B-2354; *Lactobacillus plantarum* ATCC 8014; *Bifidobacterium lactis*	22 Shiga toxin–producing *Escherichia coli* strains (STEC)	Wheat flour	*E. faecium* and *E. coli* P1 were good surrogates for the 22 STEC strains tested.	Daryaei et al., 2019
	Heat process	*Pediococcus acidilactici* ATCC 8042; *Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Enteritidis 2415, Agona F5567, Typhimurium ATCC 14028, Tennessee K4643, Newport MH57137, Heidelberg MH27651	Toasted oat cereal, peanut butter	*P. acidilactici* survived similarly to *E. faecium* in peanut butter; both survived better than the *Salmonella* cocktail. *P. acidilactici* and the *Salmonella* cocktail survived similarly in toasted oat cereal treatment above 85°C.	Deen and Diez-Gonzalez, 2019
	Moist-air convection heating	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis Phage Type (PT) 30	Almond kernels	*E. faecium* is a conservative surrogate for *Salmonella* Enteritidis PT 30 during moist-air heating.	Jeong et al., 2011
	Heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis PT 30	Almond kernels	*E. faecium* is a good surrogate for *Salmonella,* but *E. faecium* models showed higher error. Methodology, a_w, and process humidity are important parameters to monitor.	Jeong et al., 2017

Process type	Treatment	Surrogate organism	Target pathogen(s)	Matrix	Summary	References
	Heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Agona 447967, Mbandaka 698538, Montevideo 488275, Tennessee K4643, Reading ATCC 6967	Brown rice flour	*E. faecium* was a suitable surrogate at 70 and 75°C but not at 80 or 85°C.	Jin and Tang, 2019
	Hot water	*Enterococcus faecium* ATTC 8459	*Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes*	In-shell pecans	*Salmonella enterica* and *E. faecium* had the highest D-values of all tested strains. *E. faecium* was overall the most heat resistant.	Kharel et al., 2018
	Heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis PT 30	Wheat flour	*E. faecium* had similar or higher D and Z values under all parameters tested.	Liu et al., 2018
	Radio frequency heating and subsequent freezing	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis PT 30	Corn flour	*E. faecium* was a conservative surrogate for *Salmonella* under the parameters tested.	Ozturk et al., 2019
	Heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Enteritidis PT 30, Senftenberg 775W, Typhimurium, Anatum, Montevideo, Tennessee; *Listeria* strains: ATCC 15313–53 XXIII, DSMZ 20600; ATCC 49594; ATCC 35152–NCTC 7973; ATCC 13932–LMG 21264, DSMZ 27575; FRRB 2542	Confectionery formulation, chicken meat powder, pet food, savory seasoning	*E. faecium* was a suitable surrogate for all products studied except for the confectionery formulation.	Rachon et al., 2016
	Oil roasting, dry roasting	*Enterococcus faecium* ATCC 8459, *Enterococcus faecium* ATCC 35667	*Salmonella* strains: Enteritidis PT 30, Tennessee (2006/2007 peanut outbreak strain), Typhimurium TM-1, Cubana G2:229, Newport C2:e,h:1,2, Redba, Bredeney	Peanuts	*E. faecium* strains showed greater thermal resistance than all *Salmonella* strains under all parameters tested.	Sanders and Calhoun, 2014

Process type	Treatment	Surrogate organism	Target pathogen(s)	Matrix	Summary	References
	Vacuum steam pasteurization	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis PT 30, *E. coli* O157:H7	Quinoa, sunflower kernels, black peppercorns, whole flaxseed, milled flaxseed	Vacuum steam pasteurizations effectively reduced pathogens on the matrices at the parameters tested. *E. faecium* was a good surrogate for *Salmonella* PT 30 and *E. coli* O157:H7 for the matrices and parameters tested.	Shah et al., 2017
	Heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Enteritidis PT 30, Tennessee K4643, Agona 447967	Cocoa powder	*E. faecium* was a suitable surrogate at lower a_w but not at a_w above 0.45.	Tsai et al., 2019
	Radiofrequency heat	*Enterococcus faecium* NRRL B-2354	*Salmonella* Enteritidis PT 30	Wheat flour	*E. faecium* was a suitable surrogate for *Salmonella.*	Villa-Rojas et al., 2017
	Radiofrequency heat	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Agona 447967, Reading, Tennessee K4643, Montevideo 488275, Mbandaka 698538	Black peppercorns	*E. faecium* was a suitable surrogate for *Salmonella.*	Wei et al., 2018
	Radiofrequency heat	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Agona 447967, Reading, Tennessee K4643, Montevideo 488275, Mbandaka 698538	Egg white powder	*E. faecium* was a suitable surrogate for *Salmonella.*	Wei et al., 2020
	Steam treatment	*Enterococcus faecium* ATTC 8459; *Listeria innocua* ATCC 33090; *Escherichia coli* P1 ATCC BAA-1427, *Escherichia coli* K12 ATCC 23631	*Salmonella* strains: Senftenberg 775W ATCC 43845, Enteritidis PT 30 ATCC BAA-1045, Montevideo ATCC BAA-710, Thompson; *Listeria monocytogenes* strains: 4b LMG 23192, 4b LMG 23194, 1/2b LMG 26484; *Escherichia coli* O157:H7 strains: ATCC 700728, BRMSID 188, LFMFP 846	Black peppercorns	*Salmonella* was the most thermally resistant pathogen tested. *E. faecium* was a suitable surrogate for *Salmonella*.	Zhou et al., 2019

Process type	Treatment	Surrogate organism	Target pathogen(s)	Matrix	Summary	References
Thermal and Chemical	Hot water, calcium hypochlorite treatment	*Escherichia coli* strains: 080618-8, 080526-4, 080611-3, 080602-3, 080514-2	*E. coli* O157:H7strains: CR-3, MN-28, MY-29, DT-66; *Salmonella* Enteritidis strains: SE-1, SE-2 SE-3, SE-4	Mung bean seeds	Hot water treatment led to greater reductions than calcium hypochlorite treatment. Surrogate *E. coli* strains performed similarly to pathogenic strains.	Bari et al., 2009
	Product formulation: water activity and fat level, heat process	*Enterococcus faecium* NRRL B-2354	*Salmonella* Tennessee, *Salmonella* Typhimurium DT104	Peanut pastes	*Salmonella* survived in all formulations for >12 months. *E. faecium* survived at higher levels than *Salmonella* during storage.	Kataoka et al., 2014
	Ozone, heated brine and ozone	*Enterococcus faecium* OSY 31284	*Salmonella* Enteritidis ODA 99-30581-13	In-shell almonds, in-shell pistachios	*E. faecium* did not survive as well as *Salmonella* in ozone treatment on almonds or pistachios. *E. faecium* was more resistant to heat-ozone treatment than *Salmonella.*	Perry et al., 2019
Chemical	Peracetic acid	*Enterococcus faecium* NRRL B-2354	*Salmonella* strains: Newport, Senftenberg 775W, Oranienburg, Saintpaul, Typhimurium DT104	Chia seeds, flax seeds	*E. faecium* was an appropriate surrogate for *Salmonella.*	Hylton et al., 2019
	Propylene oxide (PPO)	*Enterococcus faecium* ATCC 8459; *Pediococcus acidilactici* ATCC 8042; *Staphylococcus carnosus* ATCC 51365	*Salmonella* strains: Senftenberg 775W, Montevideo 1449, Tennessee K4643, Johannesburg ARL-SE-013, Ball ARL-SE-085	Cashews, macadamia nuts	*S. carnosus* was not a suitable surrogate under the parameters tested. *E. faecium* and *P. acidilactici* were suitable surrogates under the parameters tested.	Saunders et al., 2018
Non-thermal	Intense pulsed light	*Enterococcus faecium* NRRL B-2354	*Cronobacter sakazakii* ATCC 29544	Non-fat dry milk, wheat flour, egg white powder	*E. faecium* populations were reduced less than *C. sakazakii* populations after 3 to 4 intense pulsed light treatments, for each matrix.	Chen et al., 2019
	High-intensity 405-nanometer light	*Escherichia coli* K-12 ATTC SMG 123; *Salmonella* Typhimurium Chi 3985	*E. coli* O157:H7 strains: ATCC 35150, C9990, 43894; *Salmonella* Enteritidis PT 30	Almonds	Surrogates behaved similarly to all pathogenic strains tested. Reductions were under 3 log for all processes.	Lacombe et al., 2016

[1] *Enterococcus faecium* ATTC 8459 is a clonal relative of *Enterococcus faecium* NRRL B-2354; they share over 99% sequence identity (Kopit et al., 2014).

Table 2. Studies that use surrogate organisms to study different processes in low moisture foods

Process type	Treatment	Surrogate organism	Matrix	Summary	References
Thermal	Dry-heat process	*Enterococcus faecium* NRRL B-2354	Almond meal, talc	*E. faecium* was less heat resistant in talc alone than in almond meal or almond meal with talc (used as dry-inoculum carrier); residual talc affected thermal resistance of *E. faecium*.	Ahmad et al., 2019
	Dry-heat process	*Enterococcus faecium* ATCC 8459[1], *Enterococcus faecium* ATCC 35667	Peanuts	5-log reduction of *E. faecium* was achieved using industrially relevant dry roasting parameters for peanuts.	Poirier et al., 2014
	Oven roasting, microwave roasting, oven and microwave roasting	*Enterococcus faecium* OSY31284	Peanuts	A minimum 3-log reduction of *E. faecium* was achieved for the parameters tested.	Smith et al., 2014
	Sequential infrared hot air	*Enterococcus faecium* NRRL B-2354	Pistachios	Sequential infrared hot air treatment achieved faster drying of pistachios and >5-log reduction of *E. faecium.*	Venkitasamy et al., 2017
	Sequential infrared hot air	*Enterococcus faecium* NRRL B-2354	Almonds	Sequential infrared hot air treatment achieved faster drying of almonds and varied reductions of *E. faecium.*	Venkitasamy et al., 2018
	Radiofrequency heat	*Enterococcus faecium* NRRL B-2354	Wheat flour (a_w 0.45; 22°C)	Bigelow model showed a D-value of 8.3 min at 80°C with a z-value of 11.7°C (R^2 = 0.81).	Xu et al., 2020
Thermal and Chemical	Heat with or without controlled atmosphere	*Escherichia coli* ATCC 25922	Almond powder	Heat with controlled atmosphere (low oxygen) showed increased D-values below 1°C/min heating.	Cheng et al., 2017
	Modified atmosphere storage, heat process	*Escherichia coli* ATCC 25922	Almond powder	Long-term storage at 24°C in a modified atmosphere resulted in increased reductions. Holding at 75°C for 50.4 min achieved 4-log reductions.	Cheng et al., 2018
Non-thermal	Electron beam irradiation	*Salmonella* Typhimurium LT2; *Escherichia coli* BAA-1427, BAA-1428, BAA-1430	Pecans	Irradiation under modified atmosphere conditions showed similar lethality, but reduced rancidity.	Karagöz et al., 2014

[1] *Enterococcus faecium* ATTC 8459 is a clonal relative of *Enterococcus faecium* NRRL B-2354; they share over 99% sequence identity (Kopit et al., 2014).

Validation guidelines and key references

Almond Board of California. 2014. Guidelines for using *Enterococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation. Almond Board of California, Modesto, CA.

- **The Almond Board of California's guidelines for using *Enterococcus faecium* NRRL B-2354 to validate dry-heat processes for control of *Salmonella* Enteritidis PT 30 (the pathogen of concern) on almonds.**

Anderson, D., N. Anderson, L. J. Harris, and W. Ocasio. 2017. Validation requirements in heat-processed low-moisture foods, p. 149–174. *In* R. Podolak and D. G. Black (ed.), Control of *Salmonella* and other bacterial pathogens in low-moisture foods. John Wiley & Sons, Hoboken, NJ.

- **Outlines the steps and requirements for validating heat processes for the control of pathogens in low moisture foods.**

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- **Guidelines for using *Enterococcus faecium* NRRL B-2354 to validate dry-heat processes for the control of *Salmonella* Enteritidis PT 30 (the target pathogen of concern) on pistachios.**

Chen, Y., V. N. Scott, T. A. Freier, J. Kuehm, M. Moorman, J. Meyer, T. Morille-Hinds, L. Post, L. Smoot, S. Hood, J. Shebuski, and J. Banks. 2009. Control of *Salmonella* in low-moisture foods III: Process validation and environmental monitoring. *Food Prot. Trends* 29:493–508.

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- **Describes procedures for obtaining a dry inoculum of *Enterococcus faecium* NRRL B-2354 or *Salmonella* Tennessee on talc. *E. faecium* had higher heat resistance than *Salmonella* under all parameters tested.**

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- **Outlines criteria for selecting a surrogate for process validation and lists surrogates that have been previously validated by pathogen.**

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- ***E. faecium* NRRL B-2354 and clonal relative *E. faecium* ATTC 8459 are devoid of key antibiotic resistance and virulence genes and were considered safe to use in validation studies. The two strains share over 99% sequence identity.**

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