CENTER for PRODUCE SAFETY

CPS 2013 RFP
FINAL PROJECT REPORT

Project Title
Assessing postharvest food safety risks and identifying mitigation strategies for foodborne pathogens in pistachios

Project Period
January 1, 2014 – December 31, 2015 (extended to January 31, 2016)

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Objectives
Objective 1. *To identify points during postharvest handling of pistachios where foodborne pathogens (Salmonella, E. coli O157:H7 and L. monocytogenes) may be reduced, controlled or amplified.*

> ***Objective 1a.*** *To evaluate growth or survival of foodborne pathogens on in-hull pistachios after harvest including the impact of pathogen type, temperature of exposure, harvest container (bins or trailers), time of harvest (first or second shake), and posthuller stream (floaters and sinkers).*
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> ***Objective 1b.*** *To evaluate growth or survival of foodborne pathogens on pre-dryer hulled pistachios including impact of pathogen type and post float tank stream (floater vs sinker).*
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> ***Objective 1c.*** *To evaluate the reduction of foodborne pathogens during drying under a range of drying times, temperatures and target moisture levels used by the pistachio industry.*
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> ***Objective 1d.*** *To develop a quantitative risk model to assess the parameters between the point where pistachios are shaken from the tree to the point that dehydration in the silo is complete.*

Objective 2. *To determine the impact of pistachio moisture and nut form (kernel or inshell) on the heat sensitivity of Salmonella, E. coli O157:H7, L. monocytogenes, and E. faecium inoculated pistachios.*

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Abstract
Nuts and other low-moisture foods have generally been considered low risks for foodborne illness because they are consumed in a dry state where available moisture is too low to support microbial growth. However, it is increasingly recognized that many foodborne pathogens can cause illness at very low concentrations, and outbreaks of salmonellosis and *Escherichia coli* O157:H7 gastroenteritis have been linked to consumption of a range of low moisture foods, including several tree nuts. The growth of inoculated pathogens was determined during holding of prehulled pistachios (*Salmonella*) and post-hulled pistachio floater (*Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes*) and sinker (*Salmonella*) streams. Growth curves for foodborne pathogens were determined for in-hull and hulled pistachios. Reductions of *Salmonella* achieved during the drying of pistachios were estimated. These data coupled with industry input were used to develop a model that estimates the impact of delays between harvest and hulling and between hulling and drying pistachios. Levels of *Salmonella* in sinker and, especially, in floater pistachios were influenced by delays in drying. In addition, the heat resistance of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and the surrogate *Enterococcus faecium* in inoculated pistachios under dry and moist heat conditions was characterized. The data generated in this study support *Salmonella* as the appropriate target pathogen for pistachios and provide strong evidence that thermal processes validated for this organism will reduce *E. coli* O157:H7 and *L. monocytogenes* by similar or greater levels. The data support the use of *E. faecium* NRRL 2354 as a surrogate organism for validating thermal processes in reducing *Salmonella* on pistachios.

Background
Nuts and other low-moisture foods have generally been considered low risks for foodborne illness because they are consumed in a dry state where water activity (available moisture) is too low to support microbial growth. However, it is increasingly recognized that many foodborne pathogens can cause illness at very low concentrations, such that microbial growth is not required. In the past decade, outbreaks of salmonellosis and *E. coli* O157:H7 gastroenteritis associated with consumption of a range of low moisture foods, including several tree nuts, have been documented in North America and Australia.

Salmonella is generally considered as the "pathogen of concern" in low-moisture foods, including tree nuts. However, outbreaks of *E. coli* O157:H7 gastroenteritis have been associated with flour (an ingredient in raw cookie dough), inshell hazelnuts, and walnut kernels. Since that time, *E. coli* O157:H7 and *L. monocytogenes* have been shown to survive as well as *Salmonella* when almond kernels, inshell pistachios, and walnut kernels are stored under refrigerated conditions; at ambient conditions *Salmonella* decline more slowly but *E. coli* O157:H7 and *L. monocytogenes* persist for long periods of time.

Pistachios are unique in that they are shaken directly into harvesting equipment (catch frames). Pistachios are collected into bins or trailers and transported to the huller facilities. The time between shaking the tree and hulling is influenced by the distance between the orchard and the hulling facility, volume, and huller capacity. After hulling, pistachios pass through a tank of water to separate mature nuts (sinkers) from immature nuts (floaters). Sinkers and floaters move through separate dryers, and moistures are reduced from ~40% to 9 to 14% moisture within 2 to 6 h using forced air at 160 to 200°F (processor dependent). Pistachios are further dried in silos using forced ambient air to 5 to 7% moisture for 1 to 5 days. Although not ideal, delays between hulling and drying sometimes occur.

Previous research demonstrated that there were differences in the prevalence and levels of *Salmonella* between floater and sinker pistachios. The narrow range of *Salmonella* that were isolated from pistachios suggested that several strains of *Salmonella* may have established resident and persistent populations at one or more of the participating pistachio processors.

The project aims were to understand the relative risks associated with *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* in pistachios; to elucidate the mechanisms by which floater pistachios are contaminated to a greater extent than inshell pistachios; to develop a quantitative model that will assess parameters that influence growth or survival of foodborne pathogens between the point where pistachios are shaken from the tree to the time that dehydration in the silo is complete; and to determine the impact of pistachio moisture and nut form (kernel or inshell) on the heat sensitivity of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *E. faecium* inoculated pistachios.

Research Methods and Results

Methods

Pistachios. Samples of either in-hull, hulled floaters and sinkers, or dried inshell or kernel pistachios were obtained from collaborating pistachio processors.

Bacterial cultures. The bacteria used in this study were: *Enterococcus faecium* NRRL B-2354, *Salmonella* Enteritidis PT 30 (ATCC BAA- 1045), isolated from raw almonds associated with the 2000 to 2001 outbreak; *S.* Enteritidis PT 9c (RM4635), a clinical isolate from the 2004 outbreak associated with raw almonds; *S.* Tennessee (K4643), a clinical isolate from the 2006 to 2007 outbreak associated with peanut butter; *S.* Montevideo (GRC1), isolated from pistachios associated with a 2009 recall; *S.* Saintpaul isolated from Howard Variety Walnuts and *S.* Senftenberg isolated from a pistachio survey, *E. coli* O157:H7 (Odwalla strain 223), isolated from a juice-associated outbreak; *E. coli* O157:H7 (CDC 658), a clinical isolate from a cantaloupe-associated outbreak; *E. coli* O157:H7 (EC4042), a clinical isolate from a spinach-associated outbreak; *E. coli* O157:H7 (FDA EC1738), isolated from cookie dough and *E. coli* O157:H7 phage type 4 (Health Canada NML# 11-1865), a clinical isolate from a walnut-associated outbreak, *L. monocytogenes* (4b) (LCDC81-861), isolated from raw cabbage associated with an outbreak; *L. monocytogenes* (4b), isolated from tomatoes; *L. monocytogenes* isolated from raw diced yellow onions; *L. monocytogenes,* an environmental isolate from a celery processing facility associated with an outbreak; and *L. monocytogenes*, isolated from cantaloupe associated with an outbreak.

Inoculation methods for growth studies. A rifampin-resistant cocktail of *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes* was inoculated to achieve a level of 3 log CFU/g of pistachios and incubated for up to 30 h under commercially-relevant conditions (37°C and 90% RH) to simulate a “worst case” scenario. Bacterial growth was evaluated during the incubation time by plating on tryptic soy agar and cycloheximide and CHROMagar Salmonella, CHROMagar O157 or CHROMagar Listeria. All media was supplemented with 75 µg/ml rifampin.

Inoculation methods for thermal survival studies. In-shell pistachios or pistachio kernels were inoculated at a target inoculum level of 9 log CFU/g with single strains of *E. faecium*, *Salmonella*, *E. coli* O157:H7, or *L. monocytogenes*. Inoculated pistachios were dried for 72 h under ambient conditions and then equilibrated (48–72 h) to a moisture content of 3.5 to 4%, or in some studies to 6.4%.

Results

Growth of foodborne pathogens on in-hull pistachios after harvest. Growth curves were generated for a cocktail of *Salmonella* strains inoculated onto early and late harvest in-hull pistachios and early harvest floaters and sinkers held at 90% RH and 37°C. There was no significant difference in the growth of *Salmonella* on first or second shake in-hull pistachios.

Growth of foodborne pathogens on pre-dryer hulled pistachios.

a) Impact of time of harvest. Floater and sinker pistachios were collected from a commercial pistachio processor 2 weeks apart at the beginning and middle of the harvest. No significant growth was observed in the first 3 h after inoculation. Thereafter, the growth rate of *Salmonella* on both early and middle harvest floaters was significantly ($P < 0.05$) higher than that of sinkers. Maximum levels of *Salmonella* achieved were significantly higher on middle harvest pistachios early harvest pistachios (sinkers 5.25 vs 4.30 log CFU/g; floaters: 7.30 vs 5.90 log CFU/g).

b) Impact of processor. Middle season floater pistachios were collected from three commercial processors on the same day. There was no significant growth in the first 3 h after inoculation. Thereafter, significant differences in growth of *Salmonella* were observed between processors A/B and C. Maximum concentrations were 4.77, 5.20, and 6.37 for processor A, B, and C.

c) Impact of pathogen. Late harvest floater pistachios were collected from a commercial pistachio processor. Growth of *Salmonella* and *E. coli* O157:H7 cocktails were similar; there was no significant growth in the first 3 h after inoculation. A higher maximum population was observed for *Salmonella* (6.17 vs 5.24 log CFU/g). In contrast, significant growth of *L. monocytogenes* was not observed for the first 8 h; maximum populations achieved were 4.26 log CFU/g.

d) Impact of adhering hull. Late harvest floater pistachios were collected from a commercial pistachio processor. Samples were sorted in those pistachios with wrinkled hull adhering to >25% of the shell surface and those pistachios with little (<25% of shell surface) or no adhering hull. Growth of *Salmonella* was similar for the two sets of floater pistachios for the first 8 h of incubation. At that point, little further growth of *Salmonella* was observed for those floaters with little to no adhering hull (maximum population 4.77 log CFU/g). Populations of *Salmonella* continued to increase on pistachios with adhering hull; maximum populations of 6.99 log CFU/g were observed at 30 h.

Reduction of foodborne pathogens during drying of pistachios. E. faecium-inoculated pistachios were dried on site in a drying oven with forced air (160°F) that was able to better mimic commercial drying than a laboratory oven. A 2.3 log reduction of *E. faecium* was observed within the first hour of drying (whole nut moisture fell from 30 to 18%). No further reductions of *E. faecium* were observed over the next 2 h of drying. These were similar to the data collected for commercially dried pistachios where aerobic plate counts of uninoculated pistachios fell by 2.6 log within 2 h of drying.

A quantitative model of Salmonella *growth between shaking and dehydration – Data and elements required for constructing the model.*

a) Initial *Salmonella* concentration. The concentration of *Salmonella* in freshly-harvested pistachios was unknown. Therefore, estimates were used that assumed a small portion of pistachios (100 g) was contaminated initially at one of three levels—100, 1000 or 10,000 *Salmonella* cells/g—and that these contaminated pistachios were evenly distributed in a 25,000-kg truckload of pistachios.

b) *Salmonella* growth during transportation. The lag time, growth rate and maximum populations of *Salmonella* on in-hull pistachios were determined using the data generated in the studies described above. Minimum, average, and maximum times from shaking to hulling were estimated by collaborating industry partners.

c) *Salmonella* reduction by discarding hull. Both industry data and those generated by this project were used to estimate an average of 39% of raw material that is discarded as hull. It was assumed that the same proportion of *Salmonella* would be removed during hulling.

d) Post-float tank streams. The pistachio industry estimates that, on average, 85% of the post float stream is sinkers and 15% is floaters.

e) *Salmonella* growth prior to drying. The lag time, growth rate and maximum populations on both sinkers and floaters were determined using the data generated in the studies described above. Minimum, average, and maximum times from hulling to drying were estimated by collaborating industry partners.

f) *Salmonella* reduction during drying. Data generated in this and previous studies were used to estimate average reductions 2.3 log reduction of *Salmonella* during drying. A final moisture content of 6% was estimated.

Laboratory and industry data were translated into probability distribution functions. Simulations were run using Monte Carlo simulation software (@RISK, Palisades Decision, Newfield NY). Sensitivity analysis was used to determine which data inputs have greatest influence on the model.

Floaters: The final estimated *Salmonella* concentration on the dried inshell floaters ranged from 0.000169 to 4.0710 cells/g with an average concentration of 0.5954 cell/g or 59 cells/100 g. Sensitivity analysis for the risk model showed a strong correlation between initial concentration and a weak influence of drying delay but no influence of growth during pre-hulling transportation.

Sinkers: The final *Salmonella* concentration on the dried inshell sinkers ranging from 0.000004133 to 0.1402 cells/g with an average concentration of 0.00237 cell/g or 0.2 cells/100 g. Sensitivity analysis for the risk model showed strong correlations between both initial concentration and drying delay but no influence of growth during pre-hulling transportation.

Impact of pistachio moisture and nut form on the heat sensitivity of foodborne pathogens and E. faecium *on inoculated pistachios.* Inoculated pistachios were exposed to hot oil (121°C or 127°C), hot water (80°C), and, in some cases a dry oven (138°C for 15 min). Survivors were enumerated by plating on tryptic soy agar and appropriate selective agar.

a) Strain differences – screening. In initial trials the reduction of six different strains of *Salmonella* on inshell pistachios was compared after 30 and 60 s of exposure to hot oil, hot water or an oven. In all cases, reduction of *E. faecium* was not significantly different or was lower than that of the *Salmonella* strains. The reductions among the *Salmonella* strains were not significantly different. The two pistachio isolates (*S.* Montevideo (GRC1) and *S.* Senftenberg isolated from a pistachio survey were selected for further study.

Among the *E. coli* O157:H7 strains evaluated on inshell pistachios, most declined to significantly greater extent than *Salmonella* Enteritidis PT 30 or *E. faecium*. In the hot oil and oven, declines of *E. coli* O157:H7 (Odwalla strain 223) and *E. coli* O157:H7 (Health Canada NML# 11-1865) were significantly lower than other the *E. coli* O157:H7 strains. Because the latter strain is a clinical isolate from a tree nut–associated outbreak (walnut), this strain was selected for further study.

Reductions on inshell pistachios among *L. monocytogenes* isolates were similar for all treatments and either greater than reductions of *Salmonella* Enteritidis (oil and oven) or the same as *Salmonella* Enteritidis (hot water). *L. monocytogenes* isolated from raw diced yellow onions was selected for further studies because it was associated with a more recent (2012) California recall.

b) Strain differences – survivor curves. The heat tolerance of *E. faecium* was compared to that of *Salmonella* Enteritidis PT 30, *Salmonella* Montevideo and *Salmonella* Senftenberg in hot oil (121°C) at seven time points from 0 to 4.5 min. The reduction of all organisms was similar.

Salmonella Enteritidis PT 30 survived better the most heat resistant *E. coli* O157:H7 (walnut isolate) and *L. monocytogenes (*onion isolate) observed in the screening trials. Greater than 5 log reductions were achieved on inoculated pistachios at 5, 3, and 2 min of exposure to 121°C oil for *Salmonella* Enteritidis PT 30, *E. coli* O157:H7, and *L. monocytogenes*, respectively. In contrast, very similar reductions were observed for *E. faecium*, *Salmonella* Enteritidis PT 30, *E. coli* O157:H7, and *L. monocytogenes* when inoculated pistachios were exposed to 80°C water.

c) Nut form (kernels vs inshell pistachios) and moisture. Pistachios (in-shell or kernel) were inoculated with *E. faecium* and *Salmonella* Enteritidis PT 30 and equilibrated to a moisture content of 6.4% (high moisture). Kernels were also equilibrated to 3.8% (low moisture). Pistachios were then exposed to hot oil (121°C) for 0 to 6 min. Moisture content significantly impacted the heat resistance of both *E. faecium* and *Salmonella* Enteritidis PT 30 on inoculated pistachio kernels. Greater than 5 log CFU/g reductions were observed between 3 and 4 min at 3.8% moisture compared with 2 min at 6.4% moisture. Survival of both *E. faecium* and *Salmonella* Enteritidis PT 30 were similar on inshell pistachios and kernels at 6.4% moisture and on kernels at 3.8% moisture.

Outcomes and Accomplishments

The data generated with this project specifically addressed the aims to understand the relative risks associated with *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* in pistachios; to elucidate the mechanisms by which floater pistachios are contaminated to a greater extent than inshell pistachios; to develop a quantitative model that will assess parameters that influence growth or survival of foodborne pathogens between the point where pistachios are shaken from the tree to the time that dehydration in the silo is complete; and to determine the impact of pistachio moisture and nut form (kernel or inshell) on the heat sensitivity of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *E. faecium* inoculated pistachios.

The process flow for pre-process pistachios (from harvest through storage in silo) and the associated critical points for growth of *Salmonella* (transportation, and delays in drying) is illustrated in Figure 1 (see Appendices).

Collaborating industry partners were critical for access to facilities and for providing raw materials. The data described here were presented to the pistachio industry (coordinated by the Administrative Committee for Pistachios) on a regular basis, and feedback from pistachio processors lead to modifications in data collection and analysis that significantly increased the impact of the data collected. Processors were very open and willing to provide information on how their operations work and have provided valuable feedback on the study design and to key variables in the quantitative model. Our documentation of "early" and "mid" season pistachios during and separation of float stream components in the generation of foodborne pathogen growth curves was added as a direct result of our discussions with industry representatives.

Summary of Findings and Recommendations

The data generated in this study support *Salmonella* as the appropriate target pathogen for pistachios and provide strong evidence that thermal processes validated for this organism will reduce *E. coli* O157:H7 and *L. monocytogenes* by similar or greater levels. The data support the use of *Enterococcus faecium* NRRL 2354 as a surrogate organism for validating thermal processes in reducing *Salmonella* on pistachios. A model developed with industry input and data generated in this study predicted that delays between harvest and hulling and between hulling and drying pistachios can significantly impact levels of *Salmonella* in sinker and, especially, in floater pistachios. The model predicted significantly greater levels of *Salmonella* in floaters than in sinkers.

APPENDICES

Publications and Presentations

June 23, 2015. Presentation: "*Assessing postharvest food safety risks and identifying mitigation strategies for foodborne pathogens in pistachios,*" Center for Produce Safety Produce Research Symposium, Buckhead, GA.

Budget Summary

The funds were spent as outlined in the original budget. The funds were sufficient to execute the project as proposed.

Tables and Figures

(see Figure 1 below)

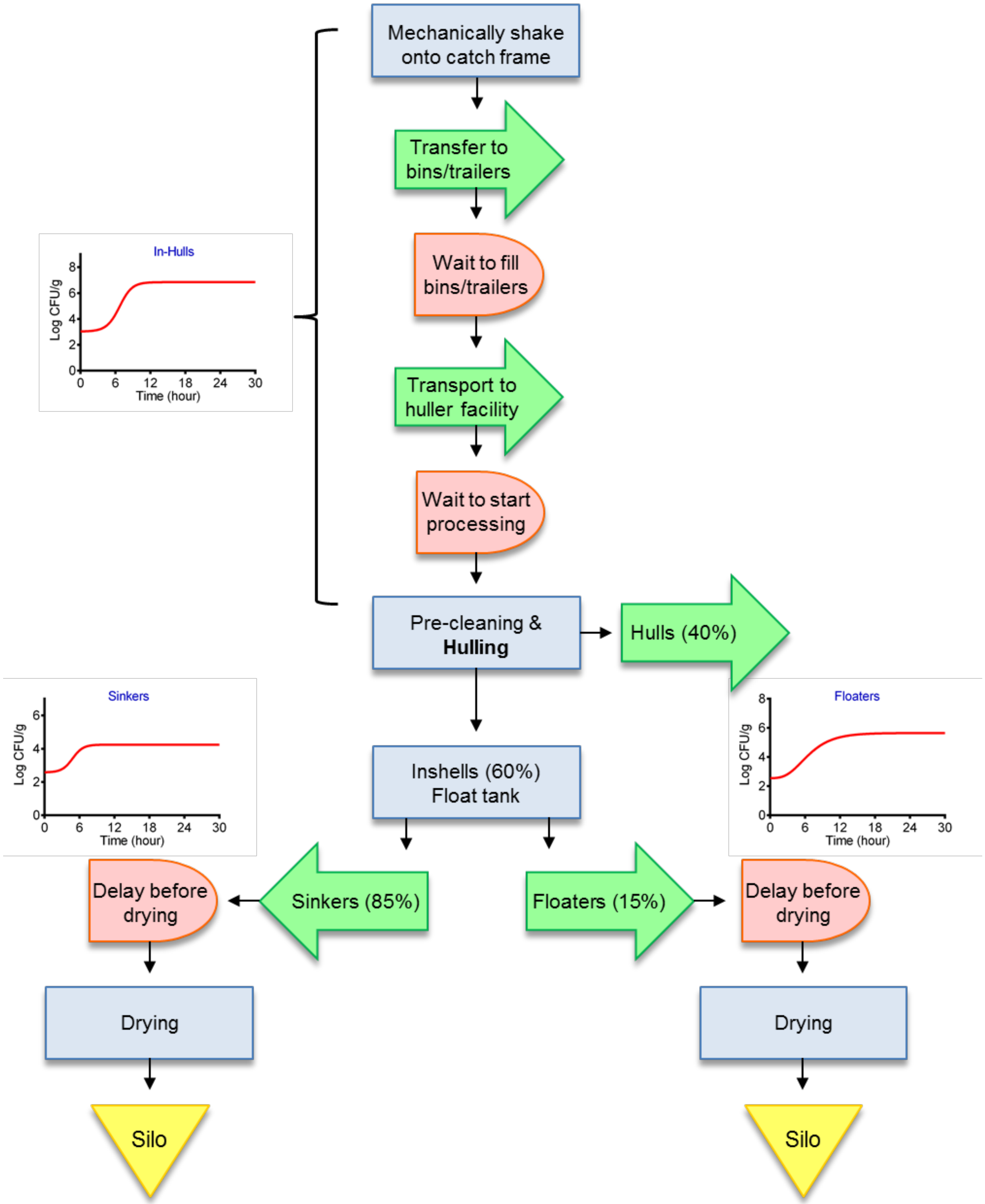

Figure 1. Process flow diagram for pre-process pistachios (from harvest through storage in silo) and associated critical points for growth of *Salmonella* (transportation, and delays in drying).