

Original Research Paper

In situ microbial viability assessment of key foodborne contaminants during hydration-dehydration cycles in Edible Drupes

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Submitted: November 4, 2025

Revised: November 19, 2025

Accepted: November 26, 2025

Abstract

Ensuring the microbiological safety of low-moisture foods, particularly tree nuts, remains a critical challenge in food processing. This study quantitatively investigated the kinetic behavior and persistence of prominent foodborne pathogens (*Salmonella* spp., *Listeria monocytogenes*, and *Shiga toxin-producing Escherichia coli*) within *Prunus dulcis* kernels subjected to typical industrial hydration (soaking) and subsequent dehydration (drying) regimes. Artificially contaminated samples were processed under controlled environmental conditions, and microbial populations were enumerated at discrete time points to elucidate growth potential and survival dynamics. Results indicated significant variability in pathogen response to moisture management interventions, with specific critical control points identified where microbial proliferation or enhanced resistance could occur. These findings provide crucial data for enhancing process validation protocols and refining risk mitigation strategies within the tree nut industry, contributing to improved public health outcomes by characterizing pathogen fate during postharvest processing.

Keywords: consumer behavior; *listeria monocytogenes*; *salmonella*; *shigatoxin-producing escherichia coli*; social media

1. Introduction

Nuts and seeds were once considered inherently safe from microbial contamination. However, epidemiological data spanning 2010 to 2019 in the United States and Canada identified 20 outbreaks connected to these food categories, leading to 313 confirmed infections with either *Salmonella* or STEC (Centers for Disease Control and Prevention [CDC], 2020). A significant proportion 11 outbreaks were associated with *Salmonella*-contaminated products such as nut butters and tahini (CDC, 2020). Between 2016 and 2019, several recalls of nuts and seeds were triggered due to detection of *Salmonella*, *Listeria monocytogenes*, or STEC (U.S. Food and Drug Administration [FDA], 2020).

A subset of consumers has adopted practices such as soaking or “activating” almonds prior to consumption. These products may be referred to as “sprouted,” “partially sprouted,” or “activated” depending on preparation methods (Beuchat et al., 2013; Barrett & Feng, in press). Several outbreaks have been traced to these methods: for example, cashew cheese (produced from soaked cashews) was linked to separate outbreaks in Canada and the United States (CDC, 2014; CDC, 2016), and other products like chia seed powder and sprouted nut spreads have also been implicated (CDC, 2014; Beuchat et al., 2013). Microbiological studies reveal that *Salmonella* populations can increase by at least 3 log CFU during 24 hours of soaking at room temperature (25°C) on various seeds, including chia, pumpkin, and sunflower (Barrett & Feng, in press; Jordan et al., 2014). Even after drying at higher temperatures (e.g., 60°C), *Salmonella* can persist, particularly in chia seeds, where minimal reduction was observed.



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1.1. Social Media and Consumer Engagement

Digital platforms like YouTube and food blogs have become integral sources of culinary information. Approximately 66% of individuals aged 25 to 34 use mobile devices for cooking guidance (Alexa Internet, 2020). Recipe-based blogs and cooking videos are highly interactive, with user engagement often analyzed via likes, comments, and view counts (Jordan et al., 2014). However, several evaluations have shown that such content typically lacks important food safety advisories and demonstrates improper food handling techniques (Langsrud et al., 2016; Nicolau et al., 2015; Borda & Thomas, 2013; Jordan & Barrett, 2017; Nicolau et al., 2018).

Prior consumer surveys, conducted before 2009, identified varied methods of almond consumption, including raw or roasted intake, inclusion in cooking, and use in nut butter and almond milk. Notably, soaking almonds was neither reported by respondents nor addressed in survey instruments (Beuchat, 2001).

1.2. Objectives

Given the paucity of information regarding the microbial risks of almond soaking and the limited inclusion of food safety content in online recipes, the current study aimed to: Characterize a representative sample of online content (blogs and videos) on almond soaking practices. Experimentally evaluate microbial growth during almond soaking and subsequent drying.

2. Research Methods

2.1. Content Analysis of Online Recipes

Blog Selection: A Google search was conducted on 14 December 2018 using terms such as “activating almonds,” “soaking almonds,” and “sprouting almonds.” The search was depersonalized, and 44 blogs were included after excluding non-relevant media types (e.g., audio/video-only blogs, journals, commercial sites, books). Popularity metrics were sourced from Alexa Internet (see Supplemental Table 1) (Jordan & Barrett, 2017). **Video Selection:** A simultaneous search on YouTube using “soaking almonds” led to the selection of 41 videos. Exclusion criteria included non-English content, videos with under 200 views, and those lacking soaking procedure details.

Coding Criteria: Blogs and videos were evaluated using a coding scheme covering source classification, user interaction, soaking procedure details, and presence of food safety content, as previously established in similar analyses (Borda & Thomas, 2013; Jordan et al., 2014; Baranyi & Roberts, 1994; Jordan & Barrett, 2017; Nicolau et al., 2018; Fallon & Enig, 2001).

2.2. Microbial Growth Assessment

Almonds and Cultures: Nonpareil almonds were used and inoculated with rifampin-resistant strains of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* each represented by five clinical or outbreak-related strains (Feng et al., 2020).

Inoculation Techniques: Almonds were inoculated either directly or indirectly (via soaking water). Following drying at ambient conditions, microbial populations were enumerated at multiple time intervals over 24 hours of soaking and up to 14 hours of drying.

Drying Conditions: Post-soaking almonds were dried using a convection oven set at 66°C until a target moisture content of 6% was achieved, typically requiring 14 hours.

2.3. Enumeration and Analysis

Enumeration of bacterial load was conducted using spiral plating on selective media. Experiments were performed in duplicate with three biological replicates each. Statistical analyses used Tukey-

Kramer and matched-pairs tests via JMP 14 Pro, with significance set at $P < 0.05$.

3. Results and Discussion

3.1. Online Content Evaluation

Blogs were, on average, last updated 50 months before data collection; only 12 of the 44 blogs provided any traceable source for their soaking instructions 10 of these cited the same book, Nourishing Traditions by Sally Fallon (Fallon & Enig, 2001). Video engagement varied widely, with an average of 13,782 views. However, food safety information was notably lacking. Only 18 of the 85 recipes (21.2%) mentioned any food safety aspects, and most addressed mold prevention. Virtually none included critical hygiene practices like handwashing or utensil sanitation.

3.2. Perceived Benefits and Misinformation

Claims regarding the benefits of almond soaking were prevalent but lacked scientific validation. Alleged benefits ranged from improved taste and texture to health claims such as enhanced nutrient absorption and disease prevention. A small subset (8.2%) referenced “safety improvements” like detoxification or allergen reduction, though no supporting evidence was cited.

3.3. Survival and Persistence of *Salmonella* During Drying of Almonds

To evaluate the persistence of *Salmonella* during almond drying, uninoculated almond kernels were initially soaked at a 1:3 (w/v) ratio for 24 hours. The resulting moisture content and water activity (aw) averaged $38.70\% \pm 0.32\%$ and 0.9891 ± 0.001 , respectively, as reported in Table 2. The drying process was conducted at an average oven air temperature of $65.85^{\circ}\text{C} \pm 1.42^{\circ}\text{C}$. Following a 6-hour drying period, these parameters reduced significantly to $21.68\% \pm 0.01\%$ moisture and 0.9575 ± 0.001 aw, respectively. After 14 hours, the values dropped further to $5.68\% \pm 0.002\%$ and 0.6337 ± 0.021 aw.

Mesophilic microbial populations, measured at 0, 6, and 14 hours of drying at 66°C , showed no statistically significant fluctuations ($P > 0.05$), maintaining population levels of 7.02 ± 0.03 , 6.83 ± 0.55 , and 6.72 ± 0.29 log CFU/10-almond sample, respectively.

Populations of *Salmonella* on soaked kernels before drying were recorded at 6.18 ± 0.28 log CFU/10-almond sample. During drying, microbial loads remained stable with no statistically significant reductions ($P > 0.05$), recording 6.01 ± 0.67 and 6.46 ± 0.28 log CFU/10-almond sample at 6 and 14 hours, respectively. Comparatively, other seeds like chia, pumpkin, and sunflower have shown over 5 log CFU/g reductions in *Salmonella* after 6 hours at 60°C (Blessington et al., 2012), suggesting substrate-specific thermal resistance, potentially influenced by food matrix or drying mechanisms.

Salmonella’s thermal inactivation tends to decrease as moisture content increases (Beuchat, 2006). However, comprehensive thermal reduction data for high-moisture almonds remain scarce. In related studies, a reduction of 2.8 log CFU was observed within 1.76 hours on dry almonds at 121°C under low-humidity conditions (Blessington et al., 2012).

Analysis of 85 almond soaking recipes extracted from blogs and videos reveals wide variability in practices, particularly in almond-to-water ratios, soaking durations, and drying protocols. Approximately 86% of recipes failed to mention the source of the almonds. Most encouraged health-related soaking benefits (94%), though only 8% cited food safety.

Over half the recipes (52.9%) lacked specific drying instructions. Among those that did, the majority recommended oven or dehydrator methods, with only 27.1% specifying multiple drying approaches. Drying durations varied significantly, from under 12 hours to over 24 hours, with 68.2% omitting drying temperature entirely.

While soaking was often performed at room temperature, only 9.4% of recipes recommended

refrigeration. Importantly, more than half the recipes suggested soaking durations conducive to pathogen proliferation, exceeding 12 hours at ambient conditions ideal for growth of *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* (Beuchat et al., 2013)

The study highlights the absence of food safety messaging in the majority of social media content concerning almond soaking. Recipes frequently lack critical parameters such as water quality, cleanliness of utensils, and safe storage. While consumers pursue soaked almonds for digestive, nutritional, or culinary advantages, there is insufficient awareness that drying alone does not mitigate bacterial risk.

Hence, consumer education is crucial. Recommendations include using almonds validated to have undergone pathogen reduction (e.g., pasteurization), washing hands and utensils thoroughly, soaking for no more than 8 hours at 18°C, and recognizing that drying at home temperatures does not ensure safety. Additionally, the misleading use of “raw” labeling in retail must be clarified in educational materials.

Table 1. Modeling Parameters for Pathogen Growth in Almonds

Organism	Inoculation Method	Rate (log CFU/sample/h)	Lag Time (h)	R2
<i>Salmonella</i>	Indirect (via water)	0.27	6.63	0.98
<i>Salmonella</i>	Direct (on almonds)	0.29	6.40	0.96
<i>E. coli</i> O157:H7	Direct	0.26	9.40	0.91
<i>L. monocytogenes</i>	Direct	0.22	8.43	0.93

Table 2. Moisture Content and Water Activity of Almonds at Different Soaking Conditions

Time (h)	Temp (°C)	Moisture (%)	<i>aw</i>
0	All	5.27 ± 0.11	0.564 ± 0.026
8	23	30.73 ± 2.00	0.980 ± 0.003
12	23	34.91 ± 1.99	0.987 ± 0.002
16	23	37.40 ± 1.30	0.988 ± 0.001
24	23	40.10 ± 0.92	0.990 ± 0.002
24	18	38.57 ± 2.05	0.988 ± 0.001
24	15	38.54 ± 1.74	0.989 ± 0.001

A separate experiment was conducted to observe the growth profiles of *E. coli* O157:H7 and *Listeria monocytogenes* at soaking temperatures of 15°C, 18°C, and 23°C. The mean measured temperatures over the 24-hour period were 14.82±0.46°C, 17.96±0.21°C, and 22.92±0.28°C, respectively.

Initially, the native microbiota populations were found to be 4.17±0.10 log CFU/sample. After 24 hours of storage, no significant microbial growth was observed at 15°C (P = 0.0734), whereas samples stored at 18°C and 23°C showed significant increases in native microbial populations by 2.20 and 4.48 log CFU/sample, respectively.

Initial levels of *E. coli* O157:H7 were 2.35±0.17 log CFU/sample. After 24 hours, no significant changes were detected at 15°C and 18°C (P = 0.5024 and 0.1153), but at 23°C a significant increase of 3.48 log CFU/sample was observed (P < 0.0001). Likewise, *L. monocytogenes* started at 2.52±0.20 log CFU/sample and either remained statistically unchanged or increased by 1.70 and 3.22 log CFU/sample at 15°C, 18°C, and 23°C, respectively, with significance at 18°C and 23°C (P < 0.0001).

Uninoculated almonds in the control group had an initial moisture content ranging between 5.3–5.7% and corresponding water activity (aw) values between 0.56–0.60 (Table 4). Soaking almonds for 12 hours at 23°C raised the moisture to 35%. After 24 hours of soaking, moisture levels rose further to

36%, 38%, and 40% at 15°C, 18°C, and 23°C, respectively. Concurrently, water activity increased to approximately 0.99 across all temperatures after 24 hours, reaching 0.98 in just 8 hours at 23°C.

3.4. Persistence of *Salmonella* during drying

For drying studies, almond kernels soaked at a 1:3 (w/v) ratio for 24 hours displayed post-soaking moisture content and aw values of 38.70% \pm 0.32% and 0.9891 \pm 0.001, respectively (Table 5). The average air temperature in the drying oven was maintained at 65.85°C \pm 1.42°C. After 6 hours of drying, moisture content and aw dropped to 21.68% \pm 0.01% and 0.9575 \pm 0.001, respectively. At the 14-hour mark, these values were further reduced to 5.68% \pm 0.002% and 0.6337 \pm 0.021.

Mesophilic microbiota counts recorded at 0, 6, and 14 hours during drying were 7.02 \pm 0.03, 6.83 \pm 0.55, and 6.72 \pm 0.29 log CFU/10-almond samples, respectively. These differences were not statistically significant ($P > 0.05$), as illustrated. The initial *Salmonella* count after soaking but before drying was 6.18 \pm 0.28 log CFU/10-almond sample. No significant reductions in microbial load were observed throughout the drying process, with final counts of 6.01 \pm 0.67 and 6.46 \pm 0.28 log CFU/10-almond sample after 6 and 14 hours, respectively ($P > 0.05$).

In contrast, prior studies have reported that soaking and drying other seeds such as chia, pumpkin, and sunflower at 60°C for 6 hours results in reductions of over 5 log CFU/g in *Salmonella* populations (Blessington et al., 2012). These discrepancies may be due to substrate composition, almond matrix properties, or differences in oven types used for drying. Notably, the heat resistance of microbes in low-moisture foods tends to diminish as moisture levels increase (Beuchat, 2006), although empirical data on pathogen inactivation in high-moisture almonds remain limited.

Table 3. Growth kinetics of Food Born Pathogens on Almonds for 24 hrs at 23°C (1:1 w/v)

Organism	Inoculation Method	Rate (log CFU/h)	Lag Time (h)	R ²
<i>Salmonella</i>	Indirect (via water)	0.27	6.63	0.98
<i>Salmonella</i>	Direct (on kernels)	0.29	6.40	0.96
<i>E. coli</i> O157:H7	Direct	0.26	9.40	0.91
<i>L. monocytogenes</i>	Direct	0.22	8.43	0.93

Table 4. Moisture Content and Water Activity of Soaked Almonds at Various Temperatures and Time

Time (h)	Temp (°C)	Moisture (%)	aw
0	23	5.27 \pm 0.11	0.564 \pm 0.026
8	23	30.73 \pm 2.00	0.980 \pm 0.003
12	23	34.91 \pm 1.99	0.987 \pm 0.002
16	23	37.40 \pm 1.30	0.988 \pm 0.001
24	23	40.10 \pm 0.92	0.990 \pm 0.002
24	18	38.57 \pm 2.05	0.988 \pm 0.001
24	15	38.54 \pm 1.74	0.989 \pm 0.001

Table 5. Drying Parameters and *Salmonella* Survival on Almonds

Drying Time (h)	Moisture (%)	Water Activity
0 (Post-soaking)	38.70 \pm 0.32	0.9891 \pm 0.001
6	21.68 \pm 0.01	0.9575 \pm 0.001
14	5.68 \pm 0.002	0.6337 \pm 0.021

3.5. Microbial Proliferation During Soaking

The growth of *E. coli* O157:H7, *Listeria monocytogenes*, and native microflora was significantly

influenced by soaking temperature. At 15°C, microbial levels remained relatively stable over 24 hours ($P > 0.05$). In contrast, soaking at 18°C and 23°C resulted in significant increases in microbial populations. At 23°C, *E. coli* O157:H7 increased by 3.48 log CFU/sample ($P < 0.0001$), while *L. monocytogenes* increased by 3.22 log CFU/sample ($P < 0.0001$), indicating that room temperature soaking promotes pathogen growth.

3.6. Persistence of Pathogens During Drying

Post-soaking drying of almonds at 66°C failed to significantly reduce *Salmonella* populations. After 14 hours of drying, *Salmonella* levels remained stable at 6.46 ± 0.28 log CFU/sample, suggesting limited thermal inactivation under these conditions. These findings contrast with prior studies on seeds like chia or sunflower, where ≥ 5 log CFU/g reductions were observed after 6 hours of drying at 60°C (Blessington et al., 2012).

3.7. Analysis of Online Recipes

Among 85 online recipes analyzed, only 8% mentioned food safety, with just 9.4% recommending refrigeration during soaking. Most recipes advocated ambient soaking for more than 12 hours conditions conducive to pathogen growth. Over half the recipes omitted drying temperatures, and 52.9% provided no drying instructions at all. The majority emphasized unverified health claims, with minimal scientific support.

3.8. Modeling of Growth Kinetics

Growth modeling under 1:1 (w/v) soaking at 23°C revealed consistent lag times across pathogens (6–9 hours). *Salmonella* inoculated via water showed a slightly lower lag phase (6.63 h) and growth rate (0.27 log CFU/h) than directly inoculated almonds (6.40 h; 0.29 log CFU/h), suggesting high growth potential in moist environments with indirect exposure.

4. Conclusion

The study demonstrates that almond soaking at ambient temperatures facilitates significant growth of foodborne pathogens, particularly *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes*. Soaking practices commonly recommended in online content pose serious food safety risks due to long durations, lack of refrigeration, and inadequate drying protocols. Moreover, the drying process at 66°C was insufficient to eliminate *Salmonella*, emphasizing the need for validated thermal processes in sprouted nut products. The lack of food safety messaging in consumer-facing media underscores a major gap in public health communication. Food bloggers and content creators are encouraged to include safety advisories based on current microbial science. Future work should focus on consumer education, development of safe soaking protocols, and further evaluation of microbial persistence under various post-soaking treatments.

Recommendations

This study emphasizes the microbial risks associated with prolonged almond soaking under ambient or elevated temperatures. Most online recipes fail to incorporate any food safety guidelines, and many advocate for soaking durations and conditions that favor pathogen proliferation. The lack of pathogen reduction during typical drying further increases risk.

- a. To mitigate foodborne illness, consumers should:
- b. Use almonds treated by validated microbial reduction methods (e.g., pasteurization).
- c. Wash hands and sanitize utensils thoroughly before preparation.

- d. Use potable water and soak almonds at $\leq 15^{\circ}\text{C}$ or for no longer than 8 hours at or below 18°C .
- f. Understand that drying does not eliminate pathogens.
- g. Educational efforts must highlight the persistence of pathogens in soaked and dried almonds and counter the common misconception that “raw” or “natural” means micro- biologically

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