

Survival of Foodborne Pathogens on Berries¹

Mary Palumbo, Linda J. Harris, and Michelle D. Danyluk²

Introduction

Fresh and frozen berries are popular foods. When berries are picked for fresh consumption, they are usually packed directly without washing because they are highly perishable. Fresh berries also are commonly included in fresh-cut fruit mixtures sold as a ready-to-eat product. Berries may be washed before freezing, but they are not usually blanched or heat-treated unless they will be used in preserves or other processed products. There is typically no “kill step” that would eliminate pathogens on fresh or frozen berries.

Foodborne illness outbreaks have been associated with the consumption of fresh or frozen berries that were contaminated with pathogenic viruses, parasites, or bacteria. Contamination can occur before or during harvest or during final preparation (Palumbo et al. 2013). The majority of outbreaks have been caused by viruses or parasites, and many of the virus-associated outbreaks have been linked to frozen berries. Outbreaks caused by viruses and parasitic coccidia are likely underreported because these pathogens are much more difficult to isolate and to study in the laboratory. Bacteria can be cultured on laboratory media, but viruses and coccidia require a host cell, often in the form of tissue culture. Norovirus, a common intestinal pathogen, cannot be grown in cell culture with currently available methods. Norovirus can be studied indirectly by using surrogate viruses or with non-culture methods that use a technique known as reverse transcription PCR.

This publication summarizes studies on the survival of pathogenic microorganisms on berries.

Outbreaks caused by viral contamination

A large norovirus outbreak occurred in Germany in 2012 when contaminated frozen strawberries were delivered by a catering firm to almost 500 schools and daycare centers (Bourquin 2012). Hepatitis A outbreaks have been associated with blueberries (Calder et al. 2003), raspberries (Ramsey and Upton 1989; Reid and Robinson 1987), and strawberries (CDC 1997a; Hutin et al. 1999; Niu et al. 1992). An outbreak of hepatitis A associated with frozen mixed berries originated in Denmark, Finland, Norway, and Sweden in October 2012; the strawberries in the mixture were eventually considered the likely cause (Nordic outbreak investigation team 2013). Viruses can survive frozen storage, and this ability contributes to geographically and temporally widespread outbreaks.

Outbreaks involving coccidian parasites

Cyclosporiasis outbreaks caused by *Cyclospora cayetanensis* have been associated with imported raspberries. A series of outbreaks occurred between 1996 and 2000 that were ultimately attributed to Guatemalan raspberries (CDC 1996, 1997b, 1997c, 1998; Herwaldt and Beach 1999; Ho

1. This document is FSHN13-12, one of a series of the Food Science and Human Nutrition Department, UF/IFAS Extension. Original publication date November 2013. Visit the EDIS website at <http://edis.ifas.ufl.edu>.
2. M. Palumbo, outreach coordinator, Western Center for Food Safety, University of California, Davis, CA 95616; L. J. Harris, cooperative extension specialist, microbial food safety, Department of Food Science and Technology, University of California, Davis, CA 95616; M. D. Danyluk, associate professor, Department of Food Science and Human Nutrition, Citrus Research and Education Center, Lake Alfred, FL 33850; UF/IFAS Extension, Gainesville, FL 32611.

et al. 2002; Murrow et al. 2002). In 2008, *C. cayetanensis* outbreaks occurred in California and Tennessee; berries were identified as the food vehicle, but the type of berry was not specified (CDC 2013; Marler-Clark 2013). *C. cayetanensis* is difficult to study because humans are its only known host. A related parasite, *Toxoplasma gondii*, which causes illness in humans and can be transmitted by food, was used in one study of survival on raspberries and blueberries (Kniel et al. 2002).

Outbreaks involving bacterial pathogens

Three outbreaks have been reported involving berries contaminated with bacterial pathogens. In 2006, five people were reported ill with *E. coli* O26, and the food vehicle was believed to be strawberries, blueberries, or a mix of both berries (Luna and Mody 2010). In 2009, a multistate outbreak of *Salmonella* Muenchen was detected when 14 people became ill from consuming contaminated blueberries (CDC 2013). And in 2011, 15 people became ill with *E. coli* O157:H7 in Oregon; matching isolates of the outbreak strain were obtained by environmental sampling in the field where the implicated strawberries were grown (Laidler et al. 2013; Oregon Public Health 2011; Terry 2011).

Available survey data

Limited survey data are available on the prevalence of pathogenic microorganisms on berries. In all cases the number of samples evaluated were too low to be statistically robust. Robertson and Gjerde (2001) determined the occurrence of parasites on various fresh fruits and vegetables available in Norway. Strawberries from Belgium, Egypt, Israel, Italy, and Norway were tested for *Cyclospora* oocysts (30 approximately 100-g samples), *Cryptosporidium* oocysts, and *Giardia* cysts (62 approximately 100-g samples for each). None of the samples were positive for *Cyclospora* or *Cryptosporidium*; two were positive for *Giardia*. Strawberries were included in a survey of produce grown in Alberta, Canada (Bohaychuk et al. 2009), and were tested by enrichment for the presence of *Salmonella* spp., *E. coli* O157:H7, and *Campylobacter* spp., and for *Cryptosporidium* spp. All 31 25-g samples were negative for the pathogens tested. Mukherjee et al. (2006) collected produce samples from conventional and organic farms in Wisconsin and Minnesota and tested for the presence of *Salmonella* spp. and *E. coli* O157:H7. A total of 126 25-gram samples of strawberries, raspberries, and blueberries (breakdown by berry not given) were examined using standard enrichment culture; none were positive for these pathogens. A survey of raspberries and strawberries from European sources

found four of 10 20-g samples of raspberries and six of 20 20-g samples of strawberries were positive for norovirus, using a reverse transcription quantitative PCR method for detection (Stals et al. 2011). The authors noted that differentiation between infectious and noninfectious virus particles was not possible with this method.

Current publication

This publication is a summary of the data on the survival of foodborne pathogens in fresh and frozen blueberries, raspberries, and strawberries (Tables 1–4). Data for other berries were not available. Although the freeze-thaw cycle may reduce pathogen viability, these laboratory and outbreak data provide clear evidence that, for many pathogens, viability and virulence are retained in frozen storage.

Survival of viruses is summarized in Tables 1–3. Prompted by the occurrence of large outbreaks of hepatitis A and norovirus associated with frozen berries, Butot et al. (2008) studied the effect of frozen storage on hepatitis A virus, norovirus, rotavirus, and feline calicivirus (used as a surrogate for norovirus) inoculated onto blueberries, raspberries, and strawberries. Freezing did not significantly reduce the viability of any of the viruses, with the exception of feline calicivirus on strawberries. Frozen storage for up to 3 months had a limited effect on survival of hepatitis A and rotavirus in the berries studied. Feline calicivirus infectivity declined on strawberries and raspberries, presumably due to their low pH. At room temperature, murine norovirus and human adenovirus decayed rapidly on strawberries but survived to the end of the shelf life of raspberries. At 4°C, no decay was observed for murine norovirus on either berry, and human adenovirus decayed only slightly (Verhaelen et al. 2012). These results illustrate the importance of using data obtained with the berry of interest when developing food safety plans.

Survival of coccidian pathogens has been evaluated on blueberries and raspberries (Tables 1 and 2). In contrast to norovirus and hepatitis A, outbreaks of cyclosporiasis have been attributed to fresh berries rather than frozen (Palumbo et al. 2013). Lee and Lee (2001) used *Eimeria acervulina*, a coccidian from the same family as *Cyclospora*, as a surrogate in studies on raspberries, and showed that infectivity was lost after freezing. Kniel et al. (2002) studied the attachment and survival of *Toxoplasma gondii* oocysts on raspberries and blueberries. In these studies, mice developed acute infections after being fed *T. gondii*-inoculated raspberries and blueberries that had been stored under refrigeration for up to 8 weeks.

Survival of *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* has been studied on intact and cut strawberries at room and refrigeration temperatures and during frozen storage (Table 4; Flessa et al. 2005; Han et al. 2004; Knudsen et al. 2001; Yu et al. 2001). No growth was observed for these pathogens on the surface of strawberries in any of the studies, though all of the pathogens were able to survive.

The information should be useful for growers and handlers of fresh berries who are developing food safety plans. The information is organized into four tables:

Table 1. Blueberries: Survival of viruses and parasites

Table 2. Raspberries: Survival of viruses and parasites

Table 3. Strawberries: Survival of viruses

Table 4. Strawberries: Growth and survival of pathogenic bacteria

References

Bohaychuk, V. M., R. W. Bradbury, R. Dimock, M. Fehr, G. E. Gensler, R. K. King, R. Rieve, and P. R. Barrios. 2009. A microbiological survey of selected Alberta-grown fresh produce from farmers' markets in Alberta, Canada. *J. Food Prot.* 72 (2): 415–420.

Bourquin, L. 2012. Strawberries implicated in massive German norovirus outbreak. Michigan State University Food Safety. Accessed April 2013. <http://michiganstateuniversityfoodsafety.wordpress.com/2012/10/07/strawberries-implicated-in-massive-german-norovirus-outbreak/>.

Butot, S., T. Putallaz, and G. Sanchez. 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *Int. J. Food Microbiol.* 126 (1–2): 30–35.

Calder, L., G. Simmons, C. Thornley, P. Taylor, K. Pritchard, G. Greening, and J. Bishop. 2003. An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiol. Infect.* 131 (1): 745–751.

Centers for Disease Control and Prevention (CDC). 1996. Update: Outbreaks of *Cyclospora cayetanensis* infection—United States and Canada, 1996. *MMWR Weekly* 45 (28): 611–612. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00043133.htm>.

Centers for Disease Control and Prevention (CDC). 1997a. Hepatitis A associated with consumption of frozen

strawberries—Michigan, March 1997. *MMWR Weekly* 46 (13): 288, 295. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00047129.htm>.

Centers for Disease Control and Prevention (CDC). 1997b. Update: Outbreaks of cyclosporiasis—United States, 1997. *MMWR Weekly* 46 (21): 461–462. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00047716.htm>.

Centers for Disease Control and Prevention (CDC). 1997c. Outbreak of cyclosporiasis—Northern Virginia-Washington, D.C.-Baltimore, Maryland, Metropolitan Area. *MMWR Weekly* 46 (30): 689–691. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00048551.htm>.

Centers for Disease Control and Prevention (CDC). 1998. Outbreak of cyclosporiasis—Ontario, Canada, May 1998. *MMWR Weekly* 47 (38): 806–809. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00055016.htm>.

Centers for Disease Control and Prevention (CDC). 2013. Foodborne Outbreak Online Database [CDC FOOD]. Accessed January 2013. <http://www.cdc.gov/foodborne-outbreaks/Default.aspx>.

Flessa, S., D. M. Lusk, and L. J. Harris. 2005. Survival of *Listeria monocytogenes* on fresh and frozen strawberries. *Int. J. Food Microbiol.* 101 (3): 255–262.

Han, Y., R. H. Linton, and P. E. Nelson. 2004. Effects of recovery, plating, and inoculation methods on quantification of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from strawberries. *J. Food Prot.* 67 (11): 2436–2442.

Herwaldt, B. L., and M. J. Beach. 1999. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. *Cyclospora Working Group. Ann. Intern. Med.* 130 (3): 210–220.

Ho, A. Y., A. S. Lopez, M. G. Eberhard, R. Levenson, B. S. Finkel, A. J. da Silva, J. M. Roberts, P. A. Orlandi, C. C. Johnson, and B. L. Herwaldt. 2002. Outbreak of cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania, 2000. *Emerg. Infect. Dis.* 8 (8): 783–788.

Hutin, Y. J. F., V. Pool, E. H. Cramer, O. V. Nainan, J. Weth, I. T. Williams, S. T. Goldstein, K. F. Gensheimer, B. P. Bell, C. N. Shapiro, M. J. Alter, and H. S. Margolis. 1999. A multistate, foodborne outbreak of hepatitis A. *New Eng. J. Med.* 340 (8): 595–602.

- Kniel, K. E., D. S. Lindsay, S. S. Sumner, C. R. Hackney, M. D. Pierson, and J. P. Dubey. 2002. Examination of attachment and survival of *Toxoplasma gondii* oocysts on raspberries and blueberries. *J. Parasitol.* 88 (4): 790–793.
- Knudsen, D. M., S. A. Yamamoto, and L. J. Harris. 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. *J. Food Prot.* 64 (10): 1483–1488.
- Kurdziel, A. S., N. Wilkinson, S. Langton, and N. Cook. 2001. Survival of poliovirus on soft fruit and salad vegetables. *J. Food Prot.* 64 (5): 706–709.
- Laidler, M. R., M. Tourdjman, G. L. Buser, T. Hostetler, K. K. Repp, R. Leman, M. Samadpour, and W. E. Keene. 2013. *Escherichia coli* O157:H7 infections associated with consumption of locally grown strawberries contaminated by deer. *Clin. Infect. Dis.* 57 (8): 1129–1134.
- Lee, M. B., and E. H. Lee. 2001. Coccidial contamination of raspberries: mock contamination with *Eimeria acervulina* as a model for decontamination treatment studies. *J. Food Prot.* 64 (11): 1854–1857.
- Luna, R. E., and R. Mody. 2010. Non-O157 Shiga toxin-producing *E. coli* (STEC) outbreaks, United States. *CDC Memo to Record*. Accessed April 2013. http://blogs.cdc.gov/publichealthmatters/files/2010/05/nono157stec_obs_052110.pdf.
- Marler-Clark, LLP. 2013. Foodborne Illness Outbreak Database. <http://outbreakdatabase.com> Accessed April 2013.
- Mattison, K., K. Karthikeyan, M. Abebe, N. Malik, S. A. Sattar, J. M. Farber, and S. Bidawid. 2007. Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *J. Food Prot.* 70 (2): 500–503.
- Mukherjee, A., D. Speh, A. T. Jones, K. M. Buesing, and F. Diez-Gonzalez. 2006. Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. *J. Food Prot.* 69 (8): 1928–1936.
- Murrow, L. B., P. Blake, and L. Kreckman. 2002. Outbreak of cyclosporiasis in Fulton County, Georgia. *Georgia Epidemiol. Rep.* 18 (1): 1–2.
- Niu, M.T., L. B. Polish, B. H. Robertson, B. K. Kanna, B. A. Woodruff, C. N. Shapiro, M. A. Miller, J. D. Smith, J. K. Gedrose, M. J. Alter, and H. S. Margolis. 1992. Multistate outbreak of hepatitis A associated with frozen strawberries. *J. Infect. Dis.* 166 (3): 518–524.
- Nordic outbreak investigation team. 2013. Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. *Euro Surveill.* 18 (27) pii: 20520.
- Oregon Public Health. 2011. Fresh strawberries from Washington County farm implicated in *E. coli* O157 outbreak in NW Oregon. Accessed September 2013. <http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2011/ucm267667.htm>.
- Palumbo, M., L. J. Harris, and M. D. Danyluk. 2013. Outbreaks of foodborne illness associated with common berries, 1983 through May 2013. Gainesville: University of Florida Institute of Food and Agricultural Sciences. <http://edis.ifas.ufl.edu/fs232>.
- Ramsay, C. N., and P. A. Upton. 1989. Hepatitis A and frozen raspberries. *Lancet* 1 (8628): 43–44.
- Reid, T. M. S., and H. G. Robinson. 1987. Frozen raspberries and hepatitis A. *Epidemiol. Infect.* 98 (1): 109–112.
- Robertson, L. J., and B. Gjerde. 2001. Occurrence of parasites on fruits and vegetables in Norway. *J. Food Prot.* 64 (11): 1793–1798.
- Stals, A., L. Baert, V. Jasson, E. Van Coillie, and M. Uyttendaele. 2011. Screening of fruit products for norovirus and the difficulty of interpreting positive PCR results. *J. Food Prot.* 74 (3): 425–431.
- Terry, L. 2011. Oregon confirms deer droppings caused *E. coli* outbreak tied to strawberries. *The Oregonian*. Accessed September 2013. http://www.oregonlive.com/washington-county/index.ssf/2011/08/oregon_confirms_deer_droppings.html.
- Verhaelen, K., M. Bouwknecht, F. Lodder-Verschoor, S. A. Rutjes, and A. M. de Roda Husman. 2012. Persistence of human norovirus GII.4 and GI.4, murine norovirus, and human adenovirus on soft berries as compared with PBS at commonly applied storage conditions. *Int. J. Food Microbiol.* 160 (2): 137–144.
- Yu, K., M. C. Newman, D. D. Archbold, and T. R. Hamilton-Kemp. 2001. Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. *J. Food Prot.* 64 (9): 1334–1340.

Table 1. Blueberries: Survival of viruses and parasites

Pathogen	Fruit product	Method of inoculation	Storage conditions	Temp (°C)	Initial counts	Storage time	Results	Unit	Comments	Reference
Feline calicivirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ 1 on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) -0.6 ± 0.09 Survival (see comment) 0.3 ± 0.15	PCR ² TCID ₅₀	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is infectious titer for treated produce	Butot et al. 2008
Hepatitis A virus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) 0.0 ± 0.18 Survival (see comment) -0.4 ± 0.19	PCR TCID ₅₀	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is infectious titer for treated produce	Butot et al. 2008
Norovirus GI and GII	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	5.1 log PCR of NV GI, 6.3 log PCR of NV GII on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival -0.9 ± 0.07 (NV GI) -0.9 ± 0.09 (NV GII)	PCR	Survival assessed by RT-PCR ³ , no method for propagating in vitro	Butot et al. 2008
Rotavirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	4.6 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) -0.8 ± 0.15 Survival (see comment) -1.2 ± 0.13	PCR TCID ₅₀	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is infectious titer for treated produce	Butot et al. 2008
<i>Toxoplasma gondii</i> VEG strain oocysts	Whole	Spot inoculation with 1 mL inoculum per berry, air dried 2 min	Refrigeration, RH not given	4	2 x 10 ⁴	2, 4, 6, and 8 weeks	All mice fed contaminated berries after storage became infected	3 mice fed at each storage time; 0 mice positive in control	Dose titration also conducted	Kniel et al. 2002

Notes to Table 1:

¹ TCID₅₀, 50% tissue culture infective dose

² PCR, PCR unit

³ RT-PCR, reverse transcription-PCR

Table 2. Raspberries: Survival of viruses and parasites

Pathogen	Fruit product	Method of inoculation	Storage conditions	Temp (°C)	Initial counts	Storage time	Results	Unit	Comments	Reference
<i>Eimeria acervulina</i> (surrogate for <i>Cyclospora cayentanensis</i>)	Whole	Berry dipped in suspension of oocysts	Freezing	-18	Expt 1: 400 oocysts Expt 2: 650 oocysts	24 h	Frozen mashed berries used as inoculum, fed to 2-day-old chicks; evaluated 5 days post inoculation for duodenal lesions and/or presence of oocysts in cecal contents or for oocysts in feces: 0/5 chicks positive in each of two experiments (5/5 chicks positive in controls)	Reported as positive or negative chicks	Freezing destroyed infectivity	Lee and Lee 2001
							Survival (see comment) -0.6	PCR ²	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is	Butot et al. 2008
Feline calicivirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) -1.1	TCID ₅₀	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is	Butot et al. 2008
							Numbers recovered declined progressively in storage, TCID ₅₀ values reduced by >2 log units			
Hepatitis A virus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) 0.0	PCR	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is	Butot et al. 2008
Human adenovirus	Whole, 25 g portion	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temp: RH 70%, 58%, and 36%, respectively	4 10 21	1 x 10 ⁶ virus particles applied	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0.1 (PCR), 0.4 (CC) 10°C: 0.2 (PCR, CC) 21°C: 0.5 (PCR), 0.3 (CC)	Mean log reduction in viral titer	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 21°C	Verhaelen et al. 2012
Murine norovirus	Whole, 25 g portion	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temp: RH 70%, 58%, and 36%, respectively	4 10 21	4 x 10 ⁵ virus particles applied	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0 (PCR, CC) 10°C: 0 (PCR), 0.5 (CC) 21°C: 0.5 (PCR), 1.1 (CC) TFL ⁴ was 3 days at 21°C; 1 log reduction not achieved at 4°C or 10°C within study time	Mean log reduction in viral titer	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 21°C and for TFL	Verhaelen et al. 2012

Pathogen	Fruit product	Method of inoculation	Storage conditions	Temp (°C)	Initial counts	Storage time	Results	Unit	Comments	Reference
Norovirus GI and GII	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	5.1 log PCRU of NV GI, 6.3 log PCRU of NV GII on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival: 0.1 (NV GI), 0.0 (NV GII) as determined by RT-PCR ⁵ ; authors report that most viruses studied were reduced by less than 1 log by freezing	PCRU	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is infectious titer for treated produce	Butot et al. 2008
Norovirus GI.4 and GI.4	Whole, 25 g portion	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temperature; RH 70%, 58%, 36%, respectively	4 10 21	8 x 10 ⁶ genomic copies of GI, 2 x 10 ⁶ copies of GII	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0 (GI, GII) 10°C: 0.4 (GI), 0.3 (GII) 21°C: 0.3 (GI), 0.2 (GII)	Mean log ₁₀ reduction in viral titer by PCR	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 21°C	Verhaelen et al. 2012
Poliovirus type 1a	Whole	1 mL virus suspension added to 60g of fruit	Refrigeration, sealed stomacher bags	4	~ 5 log TCID ₅₀	1, 4, and 9 days	No significant decline (exact counts not given)	TCID ₅₀	Raspberries severely deteriorated by day 9	Kurdziel et al. 2001
Rotavirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	4.6 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) 0.2 Survival (see comment) 0.9	PCRU TCID ₅₀	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is	Butot et al. 2008
<i>Toxoplasma gondii</i> /VEG strain oocysts	Whole	Spot inoculation with 1 mL inoculum per berry, air dried 2 min	Refrigeration, RH not given	4	2 x 10 ⁴	2, 4, 6 and 8 weeks	All mice fed contaminated berries after storage became infected	3 mice fed at each storage time; 0 mice positive in control	Dose titration also conducted	Kniel et al. 2002

¹TCID₅₀, 50% tissue culture infective dose

²PCRU, PCR unit

³CC, cell culture

⁴TFL, time required for first log₁₀-unit reduction in titer

⁵RT-PCR, reverse transcription-PCR

Table 3. Strawberries: Survival of viruses

Pathogen	Fruit product	Method of inoculation	Storage conditions	Temp (°C)	Initial counts	Storage time	Results	Unit	Comments	Reference
Feline calicivirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ ¹ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) -0.2	PCR ²	Decay of viruses calculated as log(N _x /N ₀) where N ₀ is infectious virus titer for untreated produce, N _x is infectious	Butot et al. 2008
							Survival (see comment) -2.7 TCID ₅₀ values continued slower decline because of diminishing inactivation rate	TCID ₅₀		
Feline calicivirus	Sliced	10 µl (3 x 10 ⁵ PFU)	Stored in sterile petri dishes	4 22.5 (room temp)	3.1 x 10 ³ PFU	1 to 7 days	4°C: survived 5 days, <100 at 6 days 22.5°C: <100 at 2 days	PFU	Data presented in bar graphs	Mattison et al. 2007
Hepatitis A virus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	6.2 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) 0.7	PCR	Decay of viruses calculated as log(N _x /N ₀) where N ₀ is infectious virus titer for untreated produce, N _x is infectious titer for treated produce	Butot et al. 2008
							Survival (see comment) 0.00	TCID ₅₀		
Human adenovirus	Whole, 20 to 30 g	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temperature; RH 70%, 58%, 36%, respectively	4 10 21	1 x 10 ⁶ virus particles applied	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0.6 (PCR), 0.2 (CC)	Mean log reduction in viral titer	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 21°C and for TFL	Verhaelen et al. 2012
							10°C: 0 (PCR), 1.2 (CC)			
Murine norovirus	Whole, 20 to 30 g	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temperature; RH 70%, 58%, 36%, respectively	4 10 21	4 x 10 ⁵ virus particles applied	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0 (PCR, CC)	Mean log reduction in viral titer	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 21°C and for TFL	Verhaelen et al. 2012
							10°C: 0.3 (PCR), 0.9 (CC)			
Norovirus GI and GII	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	5.1 log PCR _U of NV GI, 6.3 log PCR _U of NV GII on 15 g portions	2 days (data not shown for 30 and 90 days)	-0.1, -0.4 as determined by RT-PCR ³ (cannot be grown in cell culture)	PCR _U , 2 nd no. is NV Gill	Survival assessed by RT-PCR, no method for propagating in vitro	Butot et al. 2008
							6.3 log PCR _U of NV GI on 15 g portions			

Pathogen	Fruit product	Method of inoculation	Storage conditions	Temp (°C)	Initial counts	Storage time	Results	Unit	Comments	Reference
Norovirus GI.4 and GI.4	Whole, 20 to 30 g	3 x 100 µl inoculum applied in small drops on surface	Refrigerated incubators and room temperature; RH 70%, 58%, 36%, respectively	4 10 21	8 x 10 ⁶ genomic copies of GI, 2 x 10 ⁶ copies of GI.4	1, 2, 3, 5, and 7 days (21°C storage terminated at 3 days)	4°C: 0 (GI, GI.4) 10°C: 0.5 (GI), 0.4 (GI.4) 21°C: 1.2 (GI), 0.5 (GI.4) TFL: 21°C, 2 days (GI)	Mean log reduction in viral titer	Persistence expressed as reduction in viral titer after 7 days at 4°C and 10°C, 3 days at 2°C and for TFL	Verhaelen et al. 2012
Poliovirus type 1a	Whole	1 mL virus added to 100g fruit	Freezing, sealed stomacher bags	-20	~4.3 to 5.7 log TCID ₅₀	8 and 15 days	90% reduction in inoculated numbers by 8.4 days	TCID ₅₀		Kurdziel et al. 2001
Rotavirus	Whole, 15 g portion	Spot inoculated (50 µl over 10 spots), air dried for 60 min	Freezing for 2, 30, and 90 days	-20	4.6 log TCID ₅₀ on 15 g portions	2 days (data not shown for 30 and 90 days)	Survival (see comment) 0.7	PCRU	Decay of viruses calculated as $\log(N_x/N_0)$ where N_0 is infectious virus titer for untreated produce, N_x is infectious titer for treated produce	Butot et al. 2008

¹TCID₅₀, 50% tissue culture infective dose

²PCRU, PCR unit

³CC, cell culture

⁴TFL, time required for first log₁₀-unit reduction in titer

⁵RT-PCR, reverse transcription-PCR

Table 4. Strawberries: Growth and survival of pathogenic bacteria

Pathogen	Fruit product	pH	Method of inoculation	Treatment and/or storage conditions	Temp (°C)	Initial counts (log CFU)	Incubation time	Final counts (log CFU) or calculated decline	Unit	Comments	Reference
<i>Escherichia coli</i> O157:H7	Whole		Dip inoculated	Air dried, held in closed container	21	7.6	2 h	7.2	CFU/ berry	Results shown are for washing and membrane-transfer plating	Han et al. 2004
					4		1 day	7.3			
					4		3 days	6.8			
					4		7 days	6.0			
<i>E. coli</i> O157:H7	Whole		100 µl inoculum on surface	Air dried, held in closed container	21	7.6	2 h	7.4	CFU/ berry	Results shown are for washing and membrane-transfer plating	Han et al. 2004
					4		1 day	6.9			
					4		3 days	6.4			
					4		7 days	5.9			
<i>E. coli</i> O157:H7	Whole and cut	3.5	15 µl inoculum on surface	Dried, held in closed container	24	7.1	60 min	6.5	CFU/ sample	Results on cut berries were similar	Knudsen et al. 2001
					24		48 h	6.6			
<i>E. coli</i> O157:H7	Whole and cut	3.5	15 µl inoculum on surface	Held in closed container	5	7	7 days	Declined 2 log on whole, no decline on cut	CFU/ sample		Knudsen et al. 2001
<i>E. coli</i> O157:H7 ATCC 43895, ATCC 35150	Whole		25 µl inoculum on surface	Held in closed container	23	4.4	24 h	4.6, 3.6	CFU/g	Data also presented for injected inoculum	Yu et al. 2001
					10		3 days	2.2, 2.1			
					5		3 days	2.9, 2.4			
					-20		3 days	1.5, ≤1.00			
<i>Listeria monocytogenes</i>	Whole		Dip inoculated	Air dried, held in closed container	21	7.1	2 h	6.7	CFU/ berry	Results shown are for washing and membrane-transfer plating	Han et al. 2004
					4		1 day	6.6			
					4		3 days	5.9			
					4		7 days	5.2			
<i>L. monocytogenes</i>	Whole		100 µl inoculum on surface	Air dried, held in closed container	21	7.1	2 h	7.0	CFU/ berry	Results shown are for washing and membrane-transfer plating	Han et al. 2004
					4		1 day	6.8			
					4		3 days	6.2			
					4		7 days	6.0			
<i>L. monocytogenes</i> (5 strains)	Whole and cut		15 µl inoculum on intact surface or cut side	Air dried 1 h at room temp, held in loosely closed plastic containers	24	7.5 (high) 5.6 (low)	2 h	Whole berries: Declined 0.4 log CFU during drying, 1.0 log CFU after 48 h (high inoculum); declined 1.0 log during drying, 2.2 log after 48 h (low)	CFU/ sample	On cut berries, counts did not decrease during drying or at low inoculum level after 48 h; at high inoculum level, 0.5 log decline at 48 h	Flessa et al. 2005
					24 h						
							48 h				

Pathogen	Fruit product	pH	Method of inoculation	Treatment and/or storage conditions	Temp (°C)	Initial counts (log CFU)	Incubation time	Final counts (log CFU) or calculated decline	Unit	Comments	Reference
<i>L. monocytogenes</i> (5 strains)	Whole and cut		15 µl inoculum on intact surface or cut side	Air dried 1 h at room temp, held in loosely closed plastic containers	4	7.7 (high) 5.9 (low, whole) or 5.2 (low, cut)	1 day 4 days 7 days	Whole berries: Declined 0.7 log CFU during drying, 3 log CFU after 7 days (high); declined 1.5 log during drying, 2.7 log after 7 days (low)	CFU/sample	On cut berries, <1 log decline after 7 days at both inoculum levels	Flessa et al. 2005
<i>L. monocytogenes</i> (5 strains)	Sliced, with and without sucrose		Bags of 25 g sliced berries inoculated with 15 µl inoculum, massaged to disperse	Placed flat in single layer in freezer; samples thawed by immersing bag in 25°C water for 8 min	-20	6.7	1 day 7 days 14 days 21 days 28 days	Without added sucrose, declined 0.7 log after 1 day, 1.2 log by 28 days; with sucrose, counts remained stable over 4 weeks	CFU/sample		Flessa et al. 2005
<i>Salmonella</i> (6 serotypes)	Whole	3.5	15 µl inoculum on surface	Air dried, held in closed container	24 24	7.1 7.0	60 min 48 h	6.7 6.3	CFU/sample	No decline in numbers on cut berries	Knudsen et al. 2001
<i>Salmonella</i> (6 serotypes)	Whole and cut	3.5	15 µl inoculum on surface	Air dried 1 h at room temp, held in closed container	5	7	7 days	Declined 1 to 2 log on whole, declined 0.5 log on cut	CFU/sample		Knudsen et al. 2001