Effect of temperature and sunlight on the stability of human adenoviruses and MS2 as fecal contaminants on fresh produce surfaces

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Abstract

Determining the stability, or persistence in an infectious state, of foodborne viral pathogens attached to soft fruits and salad vegetables surfaces is essential to underpin risk assessment studies in food safety. Here, we evaluate the effect of temperature and sunlight on the stability of infectious human adenoviruses type 2 and MS2 bacteriophages on lettuce and strawberry surfaces as representative fresh products. Human adenoviruses have been selected because of their double role as viral pathogens and viral indicators of human fecal contamination. Stability assays were performed with artificially contaminated fresh samples kept in the dark or under sunlight exposure at 4 and 30°C over 24 hours. The results indicate that temperature is the major factor affecting HAdV stability in fresh produce surfaces, effecting decay between 3-4 log after 24 hours at 30°C. The inactivation times to achieve a reduction between 1 to 4-log are calculated for each experimental condition. This work provides useful information to be considered for improving food safety regarding the transmission of foodborne viruses through supply chains.

1. Introduction

Symptomatic and asymptomatic individuals excrete a wide diversity of viruses in urine or feces that are collected in urban wastewater (Cantalupo et al., 2011). Even in areas with implemented sanitation programs it represents the main vehicle for the dissemination of viral pathogens through the environment (Koopmans and Duizer, 2004; Laverick et al., 2004, Rodriguez-Manzano et al., 2010, Albinana-Gimenez et al., 2009). Despite regulations intended to assure food safety, many viral foodborne and waterborne outbreaks occur each year in developed countries. Recent foodborne outbreaks in Europe have been caused by noroviruses present in lettuce (Ethelberg et al., 2010) or HAV in semidried tomatoes (Gallot et al., 2011).

52 Pre-harvest contamination of produce by foodborne viruses can occur through a
53 variety of agents including irrigation water (Cheong et al., 2009) and natural fertilizers. In
54 addition, a further route for viral transmission to food products occurs during harvest or
55 processing by infected food handlers or by using polluted water and utensils at some point
56 of the food production chain (Carter, 2005). The pathogens associated with environmental
57 transmission routes, including water and food, encompass a wide diversity of bacteria,
58 protozoa and viruses such as Norovirus (NoV), hepatitis A virus (HAV), hepatitis E virus (HEV)
59 and adenoviruses. Thus, the use of indicators is essential for investigating water quality,
60 food safety and industrial microbiology. Some concerns have been stated regarding current
61 regulations, mainly based on bacteriologic parameters. Fecal-derived coliforms, thermo-
62 tolerant coliforms, *E. coli* and enterococci, have several drawbacks and limitations in their
63 role as indicators of fecal pollution. Various authors have concluded that these indicators
64 could fail to predict the risk for water and food-borne pathogens including viruses (Gerba et
65 al., 1979; Lipp et al., 2001). Moreover, the levels of bacterial indicators do not always
66 correlate with the concentrations of viruses, especially when these indicators are present at
67 low concentrations (Pina et al., 1998).
68 Several studies have focused on describing viruses that may be used as indicators of
69 fecal contamination in the environment and food. Human adenoviruses have been proposed
70 as an alternative indicator and as a viral index to improve the control of the microbiological
71 quality of water and food and to reduce the microbiological risk associated with medium
72 and low levels of fecal contamination in water (Puig et al., 1994; Pina et al., 1998, Formiga-
73 Cruz et al., 2003; Rodriguez-Lázaro et al., 2012). Data showing high year-round prevalence in
74 urban sewage and contaminated river water of diverse geographical areas has been
75 accumulating (Pina et al., 1998; Bofill-Mas et al., 2006; Fong et al., 2010; Kokkinos et al.,
76 2011; Rigotto et al., 2010; Simmons et al., 2011). HAdV have also been detected as the most

77 common viral pathogen in shellfish, which become contaminated by their filtration of fecally
78 polluted seawater (Formiga-Cruz et al., 2002).
79 In the environment, the stability of foodborne viral pathogens attached to soft fruits
80 and salad vegetable surfaces is an important factor for their potential transmission. Peeling
81 the surfaces from fruits and vegetables can lead to a reduction of the fecal contamination,
82 but very often they are eaten without peeling. During pre-harvest, viruses are exposed to
83 diverse environmental abiotic conditions such as temperature, sunlight, humidity and
84 desiccation. Some efforts have focused on evaluating the stability of different enteric viruses
85 exposed to different temperatures (Kurdziel et al., 2001, Croci et al., 2002) but little work
86 has been addressed to compare the relative contribution of temperature and solar
87 irradiation when combined in experimental assays. In our opinion, this information is useful
88 to improve food safety regarding the transmission of viral pathogens through the different
89 stages in food production chains.
90 The main objective of this study was to evaluate the effect of temperature and
91 sunlight as major threats to viral stability during pre-harvest and post-harvest stages. In our
92 experiments, we studied HAdV for their double role as a pathogen/indicator DNA viruses
93 (Pina et al., 1998; Formiga-Cruz et al., 2002; Rodriguez-Lazaro et al., 2012) and MS2
94 bacteriophage as an RNA virus, which has been previously used as a surrogate of
95 Noroviruses in other inactivation studies (Dawson et al., 2005). The outcomes of these
96 experiments may provide valuable information that might be considered to improve food
97 safety regarding foodborne viral pathogens.
98
99 **2. Materials and methods**
100 **2.1 Cell lines and viral stocks**
101 Human adenovirus type 2 (NCPV#00213) stocks were produced infecting A549 cells.
102 A549 were cultured in Earl's minimum essential medium (EMEM), (Gibco, Frederick, MD),

supplemented with 1% glutamine (Gibco, Frederick, MD), 50 µg of gentamycin (Gibco, Frederick, MD) per mL and 10% (growth medium) or 2% (maintenance medium) of heat-inactivated fetal bovine serum (FBS), (Gibco, Frederick, MD). Viruses were released from cells by freezing and thawing the culturing flasks for 3 times. A centrifugation step at 3000 x *g* for 20 minutes was applied to eliminate cell debris. The obtained supernatant was ultracentrifuged for 1h at 34.500 x *g* and finally resuspended in PBS, quantified and stored in 10 mL aliquots at -20ºC until used. HAdV suspensions were quantified both by qPCR and immunofluorescence assays as detailed in Sections 2.5 and 2.7. MS2 phage stocks were produced and tittered in *Salmonella* WG49 (both kindly donated by Maite Muniesa, University of Barcelona) following the ISO 10705-1.

2.2 Sunlight simulation

Light conditions applied in the assays simulated solar radiation through exposure to a 400-W Philips MSR400 HR hydrargyrum medium-arc iodide (HMI) lamp focus (Koninklijke Philips Electronics, Netherlands). As previously described, this lamp has a spectral power distribution that approximates the UVA, UVB and PAR levels of natural sunlight; a continuous spectrum from 250 to 800 nm and a power plateau from 400 to 700 nm (Dick et al., 2010). The total output radiation was controlled during our experiments using a pyranometer (LP Pyra03, Delta Ohm Srl, Italy) and UVA (320 to 400 nm) and UVB radiation (280 to 320 nm) by specific sensors for their wavelengths (LP UVA and UVB, Delta Ohm Srl, Italy). At 4 ºC, the mean values of total, UVA and UVB radiations applied in the experiments are 3.40 KW/m^2 (20.812 KJ/m^2 accumulated after 24 h), 21.91 µW/cm^2 (18.557 J/m^2) and 0.06 µW/cm^2 (51,67 J/m^2), respectively. At 30ºC, the mean values were 3.28 (20.377 KJ/m^2 accumulated after 24 h), of total radiation and 31.16 µW/cm^2 (26.867 J/m^2) of UVA. No emission in the wavelength of UVB at this temperature was registered. As a reference measure, intensities of total radiation, UVA and UVB were measured outdoors on a spring

129 sunny day at 23 ºC: they were 9.70 KW/m^2 (total radiation), 506 µW/cm^2 (UVA) and 40
130 µW/cm^2 (UVB).
131
132 **2.3 Experimental design**
133 Stability assays were performed in iceberg lettuce and strawberry samples because
134 they have been previously related to foodborne viral gastroenteritis outbreaks and their
135 surfaces present different properties. Both iceberg lettuce and fresh strawberries were
136 acquired in a local retailer and kept at 4ºC until used.
137 Lettuce and strawberry surface pieces weighting 0.5 g and 1 g respectively were cut
138 and placed on Petri plates. Then, 20 µl of viral suspensions of both HAdV2 and MS2 at a
139 concentration of 10^6 infectious units/ml were pipetted onto ten different spots on the
140 selected samples surfaces. After spiking, the inocula were dried for one hour at ambient
141 temperature in a biosecurity cabinet.
142 The temperature of the experiments was kept stable by performing the assays in
143 thermostatic chambers and monitored during the experiments. The selected temperatures
144 were 4 and 30 ºC. Dark conditions were achieved by covering the Petri plates with an
145 opaque box. All Petri plates were kept open during the assay to avoid any interference
146 between the plastic cover and the applied radiation.
147 Each experiment was performed in duplicate over 24 hours with samples taken at 0,
148 2, 4, 8 and 24 hours. At every sampling time, a Petri plate containing a piece of lettuce and
149 strawberry surface was collected from dark and light conditions. Viruses were eluted by the
150 selected protocol and viral concentrates were kept at 4 ºC until infectivity assays were
151 performed (within 24 hours). Frozen viral concentrates were kept at -80 ºC until qPCR
152 analysis.
153
154

155 **2.4 Recovery of viruses from fresh produce surfaces**

156 The recovery of infectious human adenoviruses and MS2 from lettuce and
157 strawberry was evaluated as measured by immunofluorescence and plaque forming units
158 assays, respectively, in triplicate preliminary independent experiments. Surface pieces
159 weighing 0.5 g of lettuce and 1 g of strawberry for each experimental condition were cut and
160 placed on Petri plates. A volume of 20 µl of HAdV was inoculated distributed in ten spots on
161 fresh produce surface samples at a concentration of $3x10^{10}$ genome copies per milliliter
162 (GC/ml), and the inocula were dried for one hour at ambient temperature in a biosecurity
163 cabinet.

164 Food samples were washed with 7 ml of glycine buffer (0.25 N at pH 9.5). After
165 washing, samples were briefly vortexed and stirred for 30 min at 4ºC. Finally, samples were
166 ultracentrifuged at 34500 x *g* for 1 h and pellets were suspended in 200 µl of PBS. After
167 evaluating our recovery of HAdV, following the same procedure as mentioned above,
168 triplicate experiments were also performed to test MS2 recovery from strawberry surfaces,
169 the food product with lower HAdV recoveries. The recovery values for HAdV and MS2 under
170 each condition are presented in Table 1.

171 We observed that recovery efficiency increased by addition of a homogenization
172 step with MP FastPrep®-24 Instrument (MP Biomedicals LLC.) at 4 m/s for 20 s after washing
173 the sample with 7 ml of glycine buffer (0.25 N at pH 9.5). However, for the quantification of
174 viruses in the samples at the concentrations used in this study, this step was not included in
175 the protocol.

176

177 **2.5 Evaluation of HAdV and MS2 infectivity**

178 Immunofluorescence assays (IFA) for the quantification of infectious HAdV2 were
179 performed as previously described (Calgua et al., 2011). Briefly, A549 monolayers were
180 incubated overnight in 8-well Lab-Tek II chamber slides (Nagle Nunc International,

Naperville, IL) at 37 ºC in 5% of CO_2 until they reached 90-100% of confluence. One hundred microliters of direct or diluted sample were inoculated into each well and incubated for 90 minutes at 37 ºC in 5% CO_2. After that, 2% FBS-supplemented EMEM was added and incubated for 3 days at 37 ºC in 5% CO_2. After 3 days incubation, the monolayers were fixed with chilled absolute methanol for 10 minutes and rehydrated by soaking in PBS for 5 minutes. The monolayers were blocked for 1 hour in blocking solution containing PBS with 1% Albumin from bovine serum (BSA) (w/v) and 0.05% Tween (v/v) (Panreac, SLU). After removing the blocking solution, the monolayers were incubated for 1h with their first antibody solution. After incubation, the cells were stained for 15 minutes at RT with a 1:100 dilution of goat anti-mouse IgG-FITC (Sigma-Aldrich, Steinheim, Germany) in blocking solution. The IgG antibody was removed and cells were rinsed with PBS for 15 minutes. Finally, chambers were mounted by adding UltraCruz™ mounting medium (Santa Cruz Biotechnology, Inc.) and cells were observed under UV light in an epifluorescence microscope. The theoretical detection limit of this technique is 10 viral particles/ml and even viral particles that have lost their ability to develop cytopathic effect may be still detected.

In order to quantify infectious MS2, *Salmonella* WG49 working cultures were inoculated in Tryptone-yeast extract-glucose broth (TYGB) and incubated at 36±2 ºC while shaking as specified in the ISO 10705-1. The culture absorbance was measured every 30 minutes. When an absorbance corresponding to a cell-density of approximate 10^3 cfu/ml (based on data obtained by calibration of absorbance measurements) was reached, the culture was taken from the incubator and immediately placed in melting ice to avoid the loss of F-pili by the cells. Semisolid Tryptone-yeast extract-glucose agar (ssTYGA) bottles were prepared in advance and the agar melted before the infectivity evaluation, keeping them in a water bath at 45±1 ºC. A solution of calcium-glucose, prepared with 3±0.1g of calcium chloride di-hydrate ($CaCl \cdot 2H_2O$) and 10±0.1g of glucose per 100 ml of de-ionized water, was aseptically added. Supplemented ssTYGA 2.5 ml aliquots were dispensed in capped culture

207 tubes placed in a water bath until used. To each tube, 1 ml of the sample and 1 ml of the
208 inoculum culture were added. Finally, tubes were carefully mixed and poured over the
209 surface of Tryptone-yeast extract-glucose agar (TYGA) plates, distributed and allowed to
210 solidify. Plates were incubated upside-down at 36±2 ºC for 18±2 hours.
211
212 **2.6 Nucleic acid extraction**
213 An enzymatic digestion treatment was applied in this study to reduce false positive
214 results by detection of free DNA when using qPCR analysis. Each analyzed sample was
215 digested using 100 units of Deoxyribunuclease I (DNAse I, Molecular Grade, Invitrogen),
216 according to manufactured instructions before the nucleic acid extraction. The use of a Stop
217 Buffer (provided with DNAse I) prevented excessive digestion (data not shown). Nucleic
218 acids (NA) from viral concentrates from fresh produce samples were extracted with the
219 QIAmp® Viral RNA kit (QIAgen, Inc.) using the QIAcube automated platform (QIAgen, Inc.). A
220 negative control was included in all the nucleic acid extraction procedures. Finally, NA
221 eluates were stored at -20 ºC until used.
222
223 **2.7 Quantitative real-time PCR (qPCR and RT-qPCR)**
224 HAdV quantitation was based on the qPCR assays previously described by Hernroth
225 and colleagues (2002). Standards for the real-time PCR assays for HAdV2 were produced as
226 described before (Albinana et al., 2009). For the amplification of HAdV genomes, reactions
227 were performed in a 25 µL volume with TaqMan® Environmental Master Mix 2.0 (Applied
228 Biosystems®, Foster City, CA, USA). The reaction contained 10 µL of DNA sample or 10 µL of
229 a quantified DNA plasmid, 1× TaqMan® Master Mix, and the corresponding primers and
230 TaqMan probes for HAdV. HAdV2 genomes were quantified following the activation of the
231 uracil N-glycosylase contained in the core mix (2 min at 50 °C), the activation of AmpliTaq
232 Gold for 10 min at 95 °C, and 40 cycles of amplification (15 s at 95 °C and 1 min at 60 ºC).

For the quantification of MS2, qPCR standards were produced by cloning a PCR product targeting the region coding for the maturation protein (accession number NC_001417.2) into pGEM®-T Easy (Promega, cat. No. A1360). In order to minimize contamination, NcoI enzyme was used to linearize 10 µg of DNA following supplier instructions (Promega, cat. No. R6513). All standard dilutions were then distributed into tubes and stored at -80 °C until use. We targeted a region previously described to achieve higher losses in qPCR signal after UV treatment (Pecson et al., 2009). In the qPCR reactions, we tested 5 µL of the NA extraction and of the 10-fold dilution. Amplifications were performed in 20 µL reaction mixtures with RNA UltraSense™ One-Step Quantitative RT-PCR System (Invitrogen, Carlsbad, CA, USA). The reaction contained 5 µL of sample or 5 µL of DNA standard, 1× Ultrasense Mix, 1× Ultransense Enzyme Mix, 1× ROX, plus the corresponding primer set for MS2; pecson-2F (5'-AAGGTGCCTACAAGCGAAGT-3') and pecson-2R (5'-TTCGTTTAGGGCAAGGTAGC-3') at a final concentration of 1 µM each. For this work, a TaqMan probe was designed in our laboratory; PecP-2 (5'-FAM(D-L-Probe)-ATCGTGGGGTCGCCCGTACG-BHQ1-3') and used at a final concentration of 0.25 µM. The amplification was performed following retrotranscription (60 min at 50 °C) and activation of the HotStarTaq (5 min at 95 °C), and 40 cycles of amplification (15 s at 95 °C, 1 min at 60 °C and 1min at 65 °C). Quantitations were performed with an MX3000P sequence detector system (Stratagene, La Jolla, CA, USA).

2.8 Data analyses

Viral concentrations were estimated as measured by immunofluorescence assays or plaque counts. The decay of infectious viruses was modeled as previously described (De Roda Husman et al. 2009; Tuladhar et al. 2012; Kovac et al. 2012; Verhaelen et al. 2012) by linear regression, monophasic exponential (a constant decay rate) and biphasic exponential

(two different decay rates). First, monophasic exponential decay was modelled as described by Chick (1910):

$$\log_{10}[c_t]=\log_{10}[c_0 \exp(-\lambda t)]$$

In this equation, c_t is the infectious virus concentration after a certain time (t) exposed to the specific experimental conditions. C_0 corresponds to the initial concentration and λ the constant rate of inactivation. Secondly, viral inactivation was modeled as biphasic exponential (De Roda Husman et al. 2009) according to:

$$\log_{10}[c_t]= \log_{10}[c_0 (\omega \exp(-\lambda_1 t)+(1-\omega) \exp(-\lambda_2 t)]$$

The parameter ω specifies the mixing between two decay rates and λ_1 and λ_2 are the two constants inactivation rate. The model parameters were estimated by the Least squares estimation and the best fitting model was chosen using the Aikaike Information Criterion (Hurvich and Tsai, 1989). When no significant differences were observed, the monophasic model was chosen as the more parsimonious. F-test were performed in order to assess parameter differences between experiments sharing the same kinetics model. When no significant differences were observed, the monophasic model was chosen as the more parsimonious. All analyses were performed using Prism5 (Version 5.0, GraphPad Software, 2007).

The times required to achieve from 1 to 4-log viral reduction (T_{90}-$T_{99.99}$) were estimated by solving (t) from the equations mentioned above using the most likely parameter values. The predicted (discontinuous line) and observed (individual data points) values were plotted on charts (Prism5 version 5.0 GraphPad Software, 2007), representing log inactivation [log (N_t/N_0)] versus time (Figures 1 and 2).

3. Results

3.1 Effect of temperature and sunlight on viral inactivation in fresh produce

283 The results obtained in the experiments in fresh produce indicate that temperature
284 plays an important role in the stability of HAdV whereas no significant decay due to sunlight
285 simulation was shown. In the experimental conditions evaluated, HAdV were particularly
286 stable at 4ºC and no significant inactivation ($p > 0.05$) was observed after 24 hours both in
287 lettuce and strawberry pieces placed at dark and under sunlight simulation. On the other
288 hand, at 30 ºC, the complete inactivation of infectious HAdV was achieved after 8 hours of
289 experiment both in the dark and under sunlight simulation. These results are presented in
290 Figure 1.

291 In the case of MS2, the inactivation curves are shown in Figure 2. At 4 ºC after 24 h
292 placed at dark, no significant inactivation of MS2 was observed ($p > 0.05$). Exposure to
293 artificial sunlight appears to produce a discrete effect on viral stability: only a decay of less
294 than 2-log of MS2 plaque forming units was detected on irradiated lettuce samples at 30 ºC.
295 However, this decay was not observed in strawberry samples suggesting differences in MS2
296 stability depending on fresh produce surface properties.

297 No differences in the number of genomic copies was detected in the experiments by
298 analyzing HAdV2 and MS2 by qPCR even with addition of the enzymatic pretreatments
299 applied to the samples in order to remove free DNA.

300

301 **3.2 Viral inactivation**

302 The viral inactivation of HAdV2 and MS2 at different tested conditions exhibited
303 typical first-order inactivation kinetics, excepting HAdV in strawberry at 30ºC that data sets
304 converged to a monophasic inactivation model. The correlation values (R^2) of HAdV at 30°C
305 are presented in Table 2.

306 From the results obtained at 4ºC, the decay of HAdV and MS2 observed after 24h
307 can be considered neglectable and the estimated inactivation times may exceed the times of
308 fresh produce perishability under storage conditions. The inactivation times for MS2 could

309 only be calculated in the experiments performed with lettuce at 30ºC under sunlight
310 simulation. The time to inactivate 1-log of MS2 in these conditions was 4.3 hours and to
311 inactivate 2-log was 14.67 hours. $T_{99.9}$ and $T_{99.99}$ values were not achieved in these
312 experiments. The times required to achieve a reduction from 1 to 4-log of infectious HAdV2
313 were estimated in those experiments conducted at 30ºC, both at dark and under sunlight
314 simulation and are presented in Table 2.
315 At 30ºC, under sunlight simulation the time of inactivation to achieve a decay of 4-
316 log of infectious HAdV was 24.45 hours in lettuce samples and 15.52 hours in strawberry
317 samples. At dark, the $T_{99.99}$ value was not achieved but 3-log were inactivated after 2.54
318 hours in lettuce and 2.77 hours in strawberry. From the obtained results, MS2 has shown to
319 be more stable than HAdV2. At 30ºC under sunlight simulation 4-log of infectious MS2 are
320 expected to be inactivated after 43.6 hours in lettuce and 220.9 hours in strawberry. At dark,
321 the inactivation times to achieve a decay of 4-log of the initial load were 266.1 hours in
322 lettuce and 255.7 hours in strawberry samples.
323
324 **3.3 Virus recovery from lettuce and strawberries**
325 Viral recoveries obtained in our tests with lettuce and strawberries are described in Table 1;
326 the mean recovery values for HAdV were 38.8 % and 2.5 % in lettuce and strawberry
327 respectively. The addition of a homogenization step with Fast-prep increased recoveries to
328 66.44 % for HAdV in lettuce. In strawberry samples, 8.88% recovery of HAdV and 40.67%
329 recovery of MS2 could be obtained. However, as previously indicated, this step was not
330 considered necessary for the spiked samples since the concentration of viral suspensions
331 was high enough to be detected even considering the low recoveries of the concentration
332 method.
333
334

335 **4. Discussion**

336 Foodborne viruses are not considered in current regulations, however
337 contamination by viral pathogens through the production chains has been identified and
338 viruses must be considered when defining critical control points in food safety plans. The
339 scope of this work was to evaluate the effect of temperature and sunlight on HAdV and MS2
340 inactivation times in order to identify critical scenarios for the potential foodborne
341 transmission of viral pathogens. In this study, changes in HAdV and MS2 numbers were
342 determined by infectivity assays and qPCR.

343 The results on viral stability at 4 and 30 °C at dark or under sunlight simulation show
344 temperature as the major factor directly affecting human adenovirus stability on lettuce and
345 strawberry surfaces, even when exposed to sunlight simulation. In particular, no HAdV
346 inactivation was observed at 4 ºC, at dark or under sunlight, in the tested food matrices.
347 However, at 30 ºC our results show significant reductions of HAdV on strawberry and lettuce
348 samples in 24 hours, either at dark or under sunlight radiation. Temperature has been
349 previously described as one of the most significant factors affecting pathogen inactivation
350 (Melnick and Gerba, 1980; Yates et al., 1990; Bertrand et al., 2012; Verhaelen et al. 2012).
351 While the exact mechanism behind this inactivation is uncertain, higher temperatures may
352 cause thermal degradation of the viral capsid (Yates et al., 1985). Other studies have also
353 provided evidences that viral infectivity is stable at common low temperatures of storage
354 conditions in food. A minor decay of 1-log in poliovirus infectivity was previously described
355 on lettuce and strawberry samples after 15 days at 7 ºC (Kurdziel et al., 2001). Similar results
356 were also shown for HAV on lettuce (Croci et al., 2002). Our results are consistent with those
357 presented by Verhaelen and colleagues (2012). These authors studied viral inactivation on
358 fresh produce at 4, 10 and 21ºC showing that HAdV2 remained stable at 4ºC and decayed
359 1.9-log after 3 days in inoculated strawberries at 21ºC. Overall, experimental data

360 emphasizes the idea that the infectivity of enteric viral pathogens is not challenged by those
361 low temperatures usually maintained in households to avoid bacterial growth.

362 Little information is available regarding viral stability in fresh produce exposed to
363 sunlight at different temperatures found in crops during the year. From our results, we have
364 not observed a relevant effect of sunlight on the infectivity of HAdV on fresh produce
365 surfaces. Thus, fresh produce with rough surface topographies may play a role protecting
366 viruses from sunlight inactivation.

367 In another work (Badawy et al., 1990), a comparison between the survival rates of
368 MS2, poliovirus and rotavirus was performed outdoors in winter (4-10ºC) and in summer
369 (36-41ºC). The results obtained indicated that 8 to 10 hours would be needed to achieve a
370 reduction of 2 $\log_{10}$ during the summer and 16 to 24 hours during the winter. The relative
371 contribution of temperature and sunlight to the viral inactivation was not further analyzed in
372 the study of Badawy and colleagues.

373 In the current study, we identified a different UV emission by the lamp depending
374 on the temperature set in the assay. No UV-B emission was registered in the experiments
375 performed at 30 ºC while after 24 h at 4 ºC, the samples received a mean UV-B irradiation
376 values of 51.67 J/m^2. Some studies have been performed to characterize the role of UV
377 radiation on the inactivation of viruses on food. After exposure to several doses of UV-C
378 radiation (254 nm), high decay values of hepatitis A virus, Aichi virus and feline calicivirus
379 were described on strawberries and lettuce (Fino and Kniel, 2008). In the same study,
380 inactivation was found to be greatest in lettuce and least on strawberry samples. The
381 authors considered this finding to be likely due to differences in produce surface topography
382 since lettuce has a smoother surface than strawberry. The results with MS2 in the current
383 study would agree with the data presented by Fino and Kniel (2008) and in contrast, longer
384 inactivation times for HAdV were calculated from the experiments conducted on lettuce

385 compared to those calculated for strawberry surfaces at 30 ºC under dark and solar
386 simulation irradiation.

387 From our results, 1-log of MS2 inactivation was observed on lettuce samples at 4 ºC
388 after light exposure and more than 2-log when temperature was set to 30ºC. Inactivation by
389 high temperatures appears to be more effective for HAdV than for MS2.

390 Overall, the results obtained in this work emphasize a matter of concern regarding
391 food safety. In the northern hemisphere, crops grown during winter encounter lower
392 temperatures and less sunlight hours. The results described are consistent with other
393 studies indicating that viruses are highly resistant to low temperatures. This suggests that
394 the periods with lower temperatures and less sunlight hours are likely to keep viral
395 contamination of produce infectious. When collected, fresh produce is kept cold in order to
396 avoid bacterial growth. Once again, these are the best conditions to keep viral pathogens
397 infectious through the food supply chain.

398 In crops grown at higher temperatures, it is likely that the infectivity of
399 contaminating enteric viruses will reduce over time. Particularly, a reduction of 3-log of
400 HAdV is expected after 18.23 hours on lettuce under solar radiation and 2.54 hours in the
401 dark. On strawberry surfaces, a reduction of 3-log of HAdV is expected after 5.95 hours
402 under solar radiation and 2.77 hours in the dark. These data indicate that the hours prior to
403 the harvesting of fresh produce are highly important with regard to contaminating infectious
404 enteric viral load on produce surfaces. Nevertheless, diverse susceptibility to inactivation
405 between different viruses has to be considered. The results provided in the present work
406 provide useful information to assist the identification of some critical scenarios occurring in
407 food production chains in relation to the transmission of viral pathogens.

408

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Figure 1. Comparative inactivation of HAdV on lettuce (Δ) and strawberry (■) surfaces at 4 or 30ºC at dark (A and C) or exposed to artificial sunlight (B and D). Data points represent the mean values obtained from duplicate experiments. The discontinuous lines represent the models obtained from the observed data.

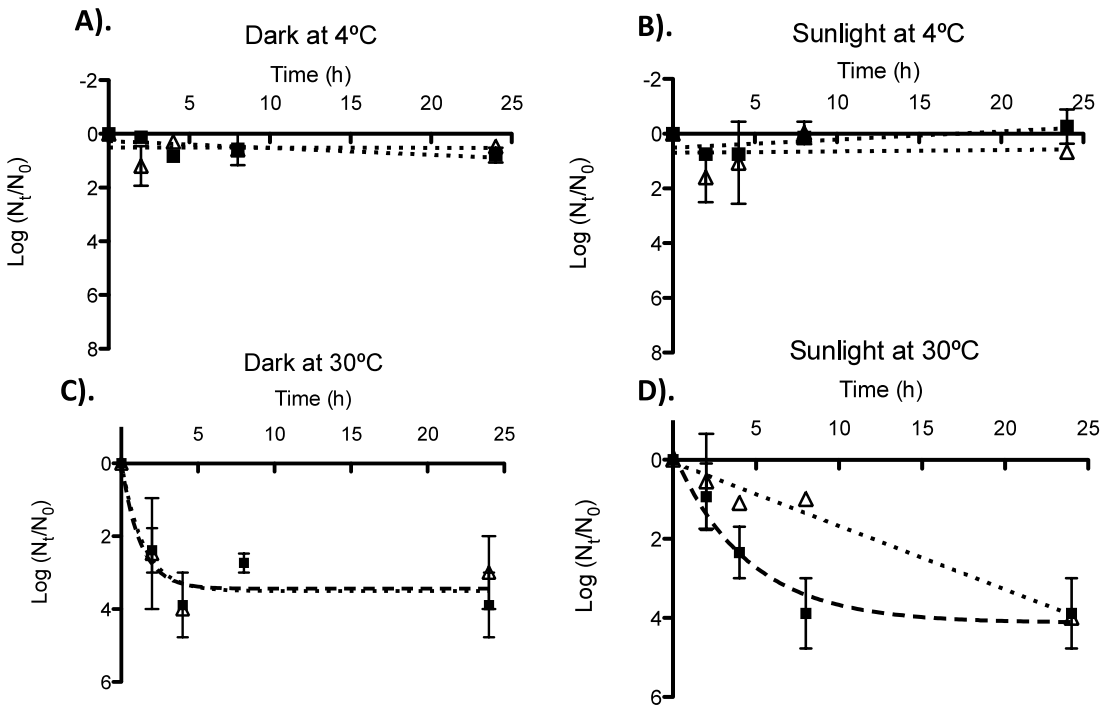

Figure 2. Comparative inactivation of MS2 on lettuce (Δ) and strawberry (■) surfaces at 4 or 30ºC at dark (A and C) or exposed to artificial sunlight (B and D). Data points represent the mean values obtained from duplicate experiments. The discontinuous lines represent the models obtained for the observed data.

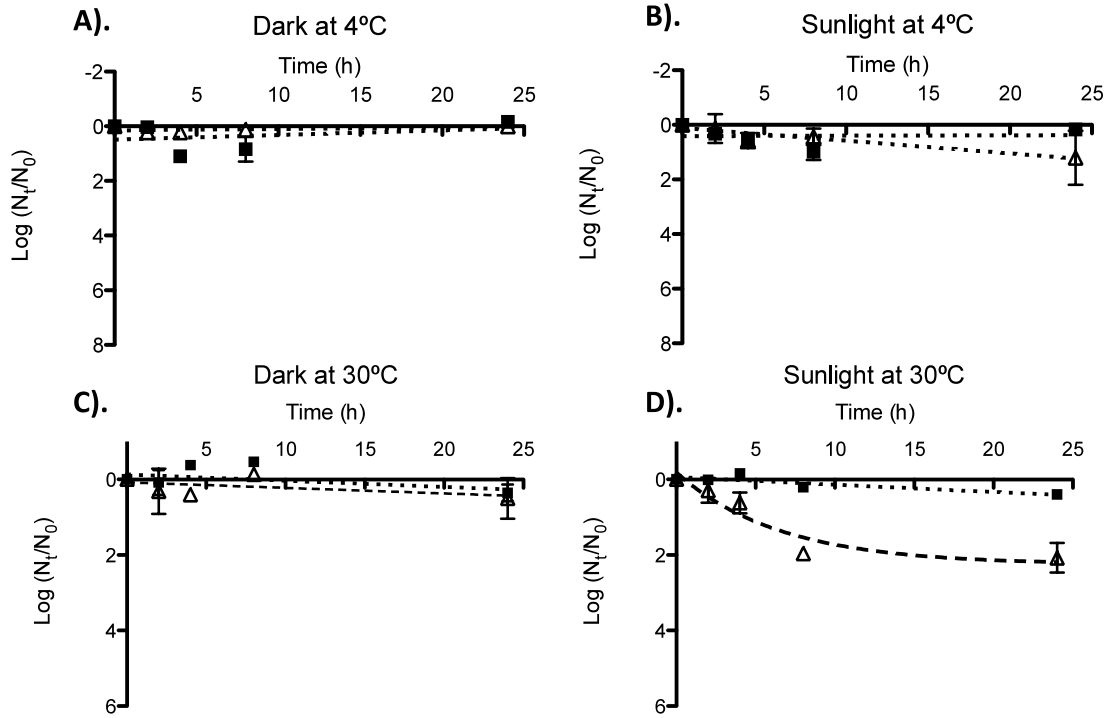

Table 1. Recovery values for HAdV and MS2 on lettuce and on strawberry samples. NT, Not tested.

	Experiment	HAdV		MS2	
	replicate	Recovery values (%)	Recovery mean (%)	Recovery values (%)	Recovery mean (%)
Lettuce	1	56.59	38.79	NT	NT
	2	19.59		NT	
	3	40.2		NT	
Strawberry	1	0.89	2.46	22.6	25.73
	2	3.04		27.6	
	3	3.46		27.0	

Table 2. HAdV inactivation parameters and times estimated from experiments developed on fresh produce surfaces at 30ºC.

Experimental conditions	Food type	Mean initial concentration (FFU/ml)	Best fitting model	R^2	λ		Inactivation times (hours)		95% CI
					Mean	95% CI			
Light	Lettuce	$1x10^4$	Lineal regression	0.91	-	-	T_{90}	5,79	1,94-9,25
							T_{99}	12,01	8,56-16,17
							$T_{99.9}$	18,23	14,35-23,93
							$T_{99.99}$	24,45	19,67-32,15
	Strawberry	$1.52x10^4$	Monophasic	0.78	0.23	0-0.48	T_{90}	1,44	0-3,26
							T_{99}	3,14	1,49-6,13
							$T_{99.9}$	5,95	3,05-NA
							$T_{99.99}$	15,53	5,72-NA
Darkness	Lettuce	$1x10^4$	Monophasic	0.66	0.81	0-2.98	T_{90}	0,43	0-2,34
							T_{99}	1,09	0-NA
							$T_{99.9}$	2,54	0-NA
							$T_{99.99}$	NA	-
	Strawberry	$1.52x10^4$	Monophasic	0.74	0.70	0-1.76	T_{90}	0,49	0-1,81
							T_{99}	1,22	0,30-2,98
							$T_{99.9}$	2,77	0,78-NA
							$T_{99.99}$	NA	-

NA, Not achieved.