This document is a postprint version of an article published in Innovative Food Science & Emerging Technologies © Elsevier after peer review. To access the final edited and published work see https://doi.org/10.1016/j.ifset.2021.102756

Document downloaded from:

1 **Highlights**

2 - Washing with chemical agents gave the highest reduction for bacteria and MNV-1.
3 - DUVC treatment was the lowest effective technology for pathogenic microorganisms.
4 - For bacteria, there was a sharp decline in the first 3 days of frozen storage.
5 - After 90 days, bacteria were not detected on the samples treated with washing treatments.
6 - MNV-1 was little affected by freezing after 180 days of frozen storage at -25 °C.

7

An innovative water-assisted UV-C disinfection system to improve the safety of strawberries frozen under cryogenic conditions

Ortiz-Solà[1], J., Viñas[1]*, I., Aguiló-Aguayo[2], I., Bobo[2], G., Abadias[2]*, M.

[1]Universitat de Lleida. Food Technology Department, AGROTECNIO-CERCA Center. Rovira Roure 191, 25198 Lleida.

[2] IRTA, Postharvest Programme. Edifici Fruitcentre, Parc Científic i Tecnològic Agroalimentari de Lleida, Parc de Gardeny, 25003 Lleida, Catalonia, Spain.

* Corresponding autor: M. Abadias (isabel.abadias@irta.cat), I. Viñas (inmaculada.vinas@udl.cat)

31 **Abstract**

32 Strawberries inoculated with *Salmonella enterica, Listeria monocytogenes* (10^8 CFU/mL, 50 µL) and
33 murine norovirus (MNV-1; 10^6 $TCID_{50}$/mL, 50 µL), were washed for 2 min in a water-assisted UV-C light
34 tank (WUVC) combined or not with 40 mg/L of peracetic acid (WUVC+PA), and 200 mg/L of free chlorine
35 solution (NaClO) with the UV-C lamps switched off. Moreover, a 'conventional' dry UV-C treatment
36 (DUVC) was also tested. After 2-min exposure, washing sanitization with chemical agents gave the highest
37 reduction for both bacteria (ca. ≥ 3.3 log CFU/g) and MNV-1 (≥ 1.8 log $TCID_{50}$/mL). DUVC treatment
38 proved to be the least effective technology (≤ 0.6 log CFU/g for bacteria and 1.5 log $TCID_{50}$/mL for MNV-
39 1). Regarding wash water, no presence of *L. monocytogenes* and *S. enterica* were reported with WUVC+PA
40 and NaClO sanitization. After disinfection, samples were frozen at -70 ± 2 ˚C in a cryogenic freezing
41 cabinet with liquid nitrogen (N_2). For both pathogens, frozen storage after washing substantially enhanced
42 their inactivation in the first 3 days (1.1-4.9 log UFC/g) compared to the reductions obtained the following
43 sampling points (0.0-0.8 log UFC/g). After 90 days, *L. monocytogenes* and *S. enterica* were not detected
44 on the samples treated with water-assisted methodologies (WUVC, WUVC+PA and NaClO treatments),
45 whilst MNV-1 was little affected. Further studies are needed to improve norovirus inactivation on frozen
46 strawberries.

47 **Industrial relevance.** The present work provides relevant information to the frozen food industry regarding
48 a suitable decontamination alternative to chlorine sanitation. Low-dose immersion-assisted UV-C allows
49 inactivation and inhibition of pathogenic microbiota while generates non-toxic byproducts and allows
50 reusing the process water, contributing to the so-called "smart green growth" attended to provide a more
51 innovative and sustainable future for the food industry.

52

53 *Keywords:* shelf-life, storage temperature, *Salmonella, L. monocytogenes*, Norovirus

54 ## 1. Introduction

55 The consumption of fruits and vegetables has increased worldwide, driven mainly by the changes
56 in the life habits of people due to a growing concern for maintaining a more balanced diet (FAO,
57 n.d; Fruitlogistica, 2020). For that reason, the consumption interests of European consumers have
58 increased the demand of fruits such as strawberries, focused in the increasingly aware of their
59 health benefits, rich in antioxidants and other biochemical compounds, which have been
60 correlated to a reduced risk of heart problems and cancer disease (Battino et al., 2017; Giamperi
61 et al., 2015; Wang et al., 2014). However, their elevated water content (ca. 90%) and high level
62 of respiration make them vulnerable to microbial growth, mainly spoilage moulds, which results
63 in a short shelf-life (1-2 days at room temperature) (Samadi et al., 2017; Tournas et al., 2006;
64 Wright and Kader, 1997). Therefore, strawberries are widely used in the food industry as an
65 ingredient in other food products or principally as a frozen fruit in many regions of Europe,
66 preventing its highly deterioration by microbial infection, with the availability to consume this
67 product year-round (Haffner, 2002). Indeed, total production of frozen berries has been gradually
68 increasing since 2015, with Europe being the largest market for frozen strawberries in the world,
69 probably due to the popularity of healthy breakfast option (such as smoothies) (Dira, 2016). In
70 2017, it has been reported that the European consumers purchased around 1.2 tons of strawberries
71 per year, being Spain in the 2nd place of the top-5 producers of frozen strawberries in Europe
72 (CBI, 2019).

73 Even though frozen strawberries are very attractive for the consumers (Janowicz et al., 2007),
74 they have been linked to human norovirus and hepatitis A virus (HAV) foodborne disease
75 outbreaks around the world in recent years (Baert et al., 2011; Bernard et al., 2014; Hjertqvist et
76 al., 2006; Le Guyader et al., 2004; Maunula et al., 2009; Sarvikivi et al., 2012; Severi et al., 2015).
77 In 2012, frozen strawberries were implicated in large-scale outbreaks of human norovirus and
78 HAV. Approximately 11,000 people in Germany were affected by human norovirus
79 gastroenteritis originated by frozen strawberries imported from China (Mäde et al., 2013) while
80 HAV in frozen mixed berries (including strawberries) from various countries (Canada, Bulgaria,

81 Serbia and Poland) was linked to an increase in cases in Northern Italy (Rizzo et al., 2013).
82 Moreover, at least 22 notifications related to the presence of viruses on berries have been reported
83 since 2018 in the RASFF portal (RASFF, 2021). Last mentioned bibliography and recent
84 publications clearly show that these viruses can survive and remain infectious after freezing
85 conditions, remaining viable for periods exceeding the shelf-life of products (Bozkurt et al., 2020;
86 Butot et al., 2008; Tavoschi et al., 2015). Survival of *Escherichia coli* O157:H7, *Salmonella* spp.,
87 and *Listeria monocytogenes* were also plausible on strawberries during refrigeration, and frozen
88 storage for at least one month (Flessa et al. 2005; Han et al. 2004; Knudsen et al. 2001; Yu et al.
89 2001). For this reason, even though low temperatures minimize the respiratory rate in fruits, and
90 the growth of pathogenic bacteria microorganisms, it is not considered as an effective mitigation
91 strategy for enteric viruses on berries (Butot et al., 2018). This problematic has led to scientists
92 and industries to find problem-solving approaches, mainly focused on introducing disinfection
93 and sanitizing methods and preservation procedures in fruit produce workflow that sufficiently
94 reduce levels of potential microbial contaminants, previous to frozen storage (Leistner, 2000).
95 Indeed, some berries (strawberries and blueberries) picked for frozen processing are usually
96 hulled in the field, transported to the processing facility, and washed with sodium hypochlorite
97 (NaClO) solutions (50 to 200 ppm) to remove debris (*e.g.*, twigs and rocks) with subsequent
98 frozen storage, improving the advantages of freezing over refrigeration in terms of food safety
99 and the extension of the fruit shelf-life (Bridges et al., 2019).

100 However, due to concerns regarding consumer and environmental safety of chlorinated washes,
101 alternative methodologies have become research focuses (Collazo et al., 2018; Goodburn et al.,
102 2013). Other authors have studied the combination of alternative disinfectants (chloride dioxide,
103 lactic acid and ozone) with freezing for the reduction of *Salmonella*, *L. monocytogenes* and *E. coli*
104 on blueberries (Bridges et al. 2019; Tadepalli et al., 2018). Previously, our research group has
105 studied the efficacy of a water-assisted short-wave ultraviolet (WUVC) technology, alone or
106 combined with peracetic acid (PA), for the reduction of natural microbiota, *L. monocytogenes* and
107 *S. enterica* in fresh strawberries (Nicolau-Lapeña et al., 2020; Ortiz-Solà et al., 2020; Ortiz-Solà

108 et al., 2021), obtaining promising results and maintaining the fruit quality. To our knowledge, this
109 combination technology has not been previously tested against enteric viruses on frozen
110 strawberry produce.

111 Since the available information of enteric virus and foodborne bacteria survival on frozen
112 produce, and the efficacy of the implementation of a washing step of current commercial
113 processes for their removal or inactivation are still lacking, the aims of the present study were (i)
114 to evaluate the efficacy of the combination of WUVC and PA coupled with an additional freezing
115 step using a cryogenic cabinet freezer operated with nitrogen (N_2), and (ii) determine the survival
116 of *Salmonella enterica, Listeria monocytogenes* and murine norovirus (MNV-1), a human
117 norovirus surrogate, on strawberries throughout one-year shelf-life at
118 frozen storage (-25 °C).

119

120 ## 2. Materials and methods

121 ### 2.1. Fruit

122 Fresh strawberries (*Fragaria* × *ananassa*) were obtained from local providers in Lleida
123 (Catalonia, Spain). Fruits were kept in trays overnight in air in the refrigerator (ERC-65, Infrico,
124 Córdoba, Spain) at 4±1 °C until use. Before the experiment, the peduncle of the fruit was carefully
125 removed by hand. Only intact, healthy and same-sized (approximately 25 g) strawberries were
126 selected.

127 ### 2.2. Microbial strains, culture conditions and fruit inoculation

128 In the present study, a cocktail of five *Salmonella enterica* subsp. *enterica* strains: Agona (ATCC
129 BAA-707; American Type Culture Collection, Manassas, USA), Michigan (ATCC BAA-709),
130 Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300;
131 *Colección Española de Cultivos Tipo*, Burjassot, Spain), and five *Listeria monocytogenes* strains:
132 serovar 1a (CECT-4031), serovar 3a (CECT-933); serovar 4d (CECT-940), serovar 4b (CECT-
133 4032) and serovar 1/2a, which was previously isolated in our laboratory from a fresh-cut lettuce
134 sample (Abadias et al., 2008), were used. *S. enterica* strains were grown individually in tryptone
135 soy broth (TSB, Biokar Diagnostics, France) medium for 20-24 h at 37 ± 1 °C. *L. monocytogenes*
136 strains were grown individually in TSB supplemented with 6 g/L of yeast extract (tryptone yeast
137 extract soy broth, TSBYE) for 20-24 h at 37 ± 1 °C. Bacterial cells were harvested by
138 centrifugation at 9800 × g, 10 min at 10 °C. Identical content of the five *S. enterica* and
139 *L. monocytogenes* were mixed to obtain a single suspension of *S. enterica* five-strain cocktail and
140 *L. monoytogenes* five-strain cocktail.

141 The day before the experiment, fresh strawberries were inoculated with a suspension containing
142 10^8 CFU/mL of *L. monocytogenes* or *S. enterica* inoculum by pipetting 50 µL in small droplets
143 on the fruit surface. Once dried (1-2 h at room temperature), strawberries were stored at 4 ± 1 °C
144 overnight. Inoculum concentration was confirmed by plating appropriate dilutions onto XLD
145 (Xylose-Lysine-Desoxycholate Agar, Biokar Diagnostics) for *Salmonella*, and onto PALCAM

146 agar (PALCAM Agar Base with selective supplement, Biokar Diagnostics) for *L. monocytogenes.*
147 The plates were incubated at 37 ± 1 °C for ca. 24 h for *Salmonella* and ca. 48 h for *L.*
148 *monocytogenes.*

149 For the virus assay, murine norovirus (MNV-1), a human norovirus surrogate, was assessed.
150 MNV-1 stocks were propagated at murine macrophage cell line RAW 264.7 (kindly provided by
151 Prof. H. W. Virgin (Washington University School of Medicine, US)). Briefly, semi-purified
152 MNV-1 virus was harvested 2 days after infection by three freeze-thaw cycles of infected cells
153 followed by centrifugation at 660 × g for 30 min to remove cell debris. RAW 264.7 cells were
154 cultured in DMEM supplemented as described in Ortiz-Solà et al. (2020) and maintained at 37 ±
155 1 °C in a 5 % CO_2 humidified incubator (NU-4950, NuAire, USA) in T175 flasks (Nunc, Thermo
156 Fisher, USA) with 85 % of relative humidity (RH).

157 Infectious viruses were enumerated by determining the 50 % tissue culture infectious dose
158 ($TCID_{50}$) with eight wells per dilution and 20 µL of inoculum per well using the Spearman-Karber
159 method (Pinto et al., 1994). Stocks of MNV-1 (1 mL) were frozen until use (-80 °C). Frozen
160 stocks were thawed and diluted one logarithmic unit (2.8×10^7 tissue culture infective dose
161 $TCID_{50}$/mL) with phosphate-buffered saline (PBS; ThermoFisher, US). Inoculation was done also
162 as described above; fruits were allowed to dry and used the same day of inoculation.

163 Prior to the experiments, the initial concentration of *S. enterica, L. monocytogenes* and MNV-1
164 in the suspensions was checked as explained below.

165 **2.3. Strawberry disinfection**

166 **2.3.1. UV-C water–assisted tank equipment**

167 All washing treatments were conducted in the UV-C water-assisted (WUVC) laboratory scale
168 equipment LAB-UVC-Gama (UV-Consulting Peschl, Castellón, Spain) equipped with 4 UV-C
169 lamps (17.2 W), an aeration and recirculation system already described in Ortiz-Solà et al. (2020).
170 Before and after each disinfection treatment, the temperature of water was measured using an
171 infrared thermometer DualTemp Pro (Labprocess distribuciones, Barcelona, Spain) and the

172 irradiance was measured through an orifice located in the lid of the equipment using a UV-sensor
173 EasyH1 (Peschl Ultraviolet, Mainz, Germany).

174 **2.3.2. Sanitizing treatments**

175 For each sanitization treatment, 20 strawberries (25-g each, approximately) were used. Samples
176 were washed for 2 min using the WUVC equipment in combination with 40-mg/L (ppm) peracetic
177 acid (PA) (WUVC+PA), since previous work reported that this combination significantly reduced
178 pathogenic microorganisms on strawberries (Nicolau-Lapeña et al., 2020). Moreover, WUVC
179 alone and 200 ppm of sodium hypochlorite (NaClO) (pH 6.5) were also added as control
180 treatments in the same equipment commented above. For WUVC and WUVC+PA treatments,
181 lamps were preheated during 10 min until an irradiance of 10.5 ± 0.5 W/m^2 was achieved. The
182 tank was filled with 12 L of cold tap water (8 ± 2 °C) with or without PA and recirculation and
183 aeration system were switched on. Treatment time was set up for 2 min, corresponding to an
184 irradiation dose of 1.3 kJ/m^2. For NaClO treatment, the process was set up at the same conditions
185 except that the lamps were switched off. After NaClO treatment strawberries were rinsed in tap
186 cold water for 2 min to eliminate any residual. The free chlorine concentration was checked with
187 an ion specific meter Hanna Instruments HI 95734-11 (Rhode Island, US) and PA concentration
188 was determined by iodometric titration with potassium permanganate and sodium hydroxide
189 (NaOH) 2M (Panreac AppliChem, Barcelona, Spain). Furthermore, pH and ORP (Oxidation
190 Reduction Potential) values were measured using pH meter (Crison GLP-22, Barcelona, Spain).
191 After the washing treatments, strawberries were let at room temperature to drain the excess of
192 water (1-2 h at 22°C).

193 Moreover, one-sided dry UV-C (DUV) treatment without water immersion during 2 min was also
194 tested (1.3 kJ/m^2) in an UV-C light cabinet, in order to compare it with the novel water-assisted
195 UV-C technology. A batch of inoculated strawberries was left untreated (control, CK) for the
196 comparison throughout the experimental time.

197

198 ### 2.4. Strawberry cryogenic freezing process and storage conditions

199 Once dried, three strawberries per treatment (n=3) were packaged under polypropylene (PP) trays
200 (375 mL) sealed with tray-lidding film by a self-sealing lab scale equipment (AK-RAMON TS-
201 150, Barcelona, Spain). Packaged samples were frozen at -70 ± 2 ˚C in a cryogenic freezing
202 cabinet which operates by injecting liquid Nitrogen (N_2) inside (Carburos Metálicos-Air Products
203 Group Batch Freezer CM-85/1090, Carburos Metálicos SA, Barcelona, Spain, Figure 1). With
204 this equipment, freezing of fruits is done in three phases: (i) cooling from the initial fruit
205 temperature to freezing point with freezing temperature (FT) (without crystal ice formation); (ii)
206 super-cooling with a transition phase time with a remaining constant temperature (this is the phase
207 in which most of the ice forms); (iii) super-chilling when the temperature falls for the mixture of
208 water and ice, cooling down to a final temperature of approximately -20 °C (Freezing time
209 (Ftime)). This methodology allows a smaller crystal formation, so cell integrity is maintained and
210 water is better retained after thawing, offering potential benefits for the preservation of fresh foods
211 (Comandini et al., 2013; Stonehouse and Evans, 2015; Sun et al., 2019). In the present study, all
212 trays including three strawberries per tray were first pre-cooled to approximately 0-5 °C in the
213 cryochamber commented above. Subsequently, strawberries were gradually cooled down to
214 approximately -1.5 °C by the injection of liquid N_2 (phase 2). During this cooling process,
215 strawberries took the supercooling state. Finally, strawberries were frozen with a temperature of
216 approximately -70 ± 2 °C, to resolve the supercooled state and freeze strawberries completely (-
217 20 °C). Throughout the whole freezing process, the internal temperature of the fruit was recorded
218 by placing the probe of the data logger JUMO TDA-300/TDA-3000 (Berlin, Germany) in the
219 interior of a single fruit. After freezing, packaged samples were stored at -25.0 ± 0.5 °C for 12
220 months.

221 ### 2.5. Microbiological analysis

222 Populations of *S. enterica*, *L. monocytogenes* and the infectivity of MNV-1 were evaluated before
223 and after sanitation process and after 3, 90, 180 and 360 days of storage at -25 °C. Before and
224 after each treatment, three strawberries were randomly taken and weighed. During storage, the

225 day before each sampling time, three strawberries from the same tray (n=3) were taken. Each
226 strawberry was placed in an individual sterile filter bag (80 mL BagPage®, Interscience
227 BagSystem, Saint Nom, France) and samples were thawed at 4 ºC overnight. Fresh strawberries
228 or defrost strawberries and exudates were diluted with buffered peptone water 1:4 (w:v) and
229 mashed in a paddle blender (MiniMix, Interscience, France) for 2 min at 9 strokes/s. Aliquots of
230 the mixture were serially diluted in saline peptone (SP), plated (0.1 or 1.0 mL for fresh and frozen
231 strawberries, respectively) in duplicate onto XLD for *S. enterica* and PALCAM agar for *L.*
232 *monocytogenes* determination. Plates were incubated at 37 ± 1°C for 24 h and 48 h, respectively.
233 Results were calculated as colony forming units per g (CFU/g) and expressed as log CFU/g.
234 Frozen strawberries were left to thaw in the refrigerator (10 °C) overnight in sterile bags in order
235 to maintain the exudates. Strawberries and their exudates were analysed as previously described.
236 For the samples analyzed after the decontamination and freezing steps, detection limit was 1.30
237 log CFU/g whilst that of samples during storage (90, 180 and 360 days) was 0.70 log CFU/g (5
238 CFU/g). Logarithmic reductions of the pathogens due to the washing treatments were calculated
239 by the following equation (Eq. 1):

240 $$\text{Log reductions (Log CFU/g)} = \text{Log}(N_0) - \text{Log}(N_t) \qquad \text{Eq. 1}$$

241 Where N_0 is the mean of the initial population, and N_t is the population at each sampling time
242 (CFU/g).

243 Population of bacteria were also determined in wash water after the sanitation treatments.
244 Duplicate 100 µL samples were plated in XLD and PALCAM as indicated above. In parallel,
245 duplicate 1-mL samples were added to 9-ml Dey-Engley tubes. Results were expressed as log
246 CFU/mL. When counts were below the limit of detection (50 CFU/mL), and presence was
247 confirmed by Dey-Engley colour variation followed by streaking onto XLD or PALCAM, an
248 arbitrary value of ½ limit of detection (25 CFU/mL) was attributed.

249 For MNV-1 determination, extraction of the virus from the treated samples (n=3) was performed
250 as previously described (Ortiz-Solà et al. 2020). Briefly, confluent RAW 264.7 cells with

251 supplemented DMEM 10 % were transferred to 96-well microtiter plates (ThermoFisher, USA).
252 Micro-plates were stored at 37 ± 1 °C in a 5 % CO_2 and 85 % of humidity relative (HR) conditions
253 during 24 ± 2 h. Afterwards, DMEM 10 % was removed and 20 µL of the tenfold dilutions with
254 PBS of each extracted sample were inoculated into 8 wells of a 96-well microtiter plates of
255 confluent RAW 264.7. Plates were incubated at same conditions commented above. After 1 h
256 incubation, 150 µL/well of DMEM supplemented with 2 % FBS were added and incubated for
257 2–3 days at 5 % CO_2 and 85 % of HR. Then, RAW 264.7 monolayers with cytotoxicity effects
258 were observed by visual examination using the optical inverse microscope. MNV- 1 positive
259 sample was diluted one log in PBS (2 M $NaNO_3$, 1 % beef extract, and 0.1% Triton X-100) and
260 used as norovirus control. Negative controls were studied using PBS.

261 The MNV-1 infectivity of each treated strawberry was calculated by determining $TCID_{50}$ with 8-
262 wells per dilution and 20 µL of inoculum/well. The number of wells with cytopathic effect were
263 documented. The reduction of the infectivity was calculated as log (N_0/N_t), where N_t is the
264 infectious virus titer after each treatment and N_0 is the initial virus infect titer found in untreated
265 strawberries (Falcó et al., 2018).

266 **2.6. Statistical analysis**

267 All data were analysed for significant differences by applying analysis of variance test (ANOVA).
268 The criterion for statistical significance was $P < 0.05$ with the Tukey's Honest Significant
269 Difference (HSD) test to evaluate the differences during storage conditions after the disinfection
270 treatments. All statistical analyses were carried out using JMP PRO 14.0.1 (SAS Institute Inc.,
271 Cary, USA). MNV-1 experiment was repeated twice with 3 replications (n=6).

272

273 ## 3. Results and discussion

274 ### 3.1. Foodborne pathogenic bacteria inactivation on strawberries and wash water after
275 disinfection treatments

276 Parameters of the water used to wash strawberries were analysed during each sanitization
277 treatment (Table 1). There were no differences between pH, ORP, peracetic acid (PA) and
278 chlorine concentration after 2 min treatment (data not shown). Regarding the effect of the assayed
279 technologies on microbial populations in the process water after washing for 2 min, foodborne
280 bacterial pathogens were only found in the water-assisted UV-C (WUVC) treatment without PA,
281 persisting in populations of <1.0 log CFU/mL for both microorganisms. On the other hand, no
282 presence of *L. monocytogenes* and *S. enterica* were reported in wash water after the WUVC
283 combined with PA at 40 ppm (WUVC+PA), and after chlorine (NaClO) sanitization at 200 ppm.
284 Undoubtedly, bacterial cells that are washed off from the fruit product are inactivated by the
285 sanitizer present in the wash solution, thereby reducing the risks for cross contamination. The
286 three-way action for disinfecting the produce by the synergistic effect of integrated strategies
287 involving UV-C light, PA, and the simultaneous physical movement of the wash water could
288 account for the higher efficacy against foodborne pathogens in washing water and fruit (as could
289 be seen below). Compared to other wash water disinfectants, PA has less potential of producing
290 degradation by-products, which are easily dissolved in water and non-toxic, thus making this
291 sanitizer a good alternative to chlorine (Banach et al., 2015).

292 On strawberries, initial counts of *S. enterica* and *L. monocytogenes* population were 5.7 ± 0.4 and
293 5.6 ± 0.2 log CFU/g, respectively (Fig. 2A and 2B). After 2-min exposure to disinfection
294 treatments, NaClO sanitization and the combination of WUVC+PA gave the highest reduction of
295 both bacteria (ca. ≥ 3.3 log CFU/g). On the other hand, the non-immersed dry UV-C (DUVC)
296 technology (1.3 kJ/m^2) gave reductions about 0.4 and 0.6 log CFU/g for *S. enterica* and *L.*
297 *monocytogenes*, respectively, being equivalent to the population obtained with the untreated
298 samples (CK) ($P > 0.05$). This lower inactivation observed with air-transmitted DUVC treatment
299 were in concordance with previous investigation, reporting < 1 log reduction of *E. coli* O157:H7,

300 *S. enterica* and *L. monocytogenes* on fresh strawberries after 120 s exposure (13.3 kJ/m^2) of
301 DUVC (Butot et al., 2018). On the other hand, bacterial population were reduced to 2.6 and 2.2
302 log for *L. monocytogenes* and *S. enterica* when strawberries were washed with WUVC alone,
303 while the addition of 40 ppm of PA increased the reduction range to 1.0 and 2.0 log CFU/g,
304 respectively. However, there were no significant differences between them ($P > 0.05$). Therefore,
305 results confirmed the effectiveness of the different washing treatments, since the WUVC and the
306 combination treatment (WUVC+PA) are comparable to disinfection with free chlorine ($P > 0.05$),
307 allowing the inhibition of the principal foodborne bacterial pathogens on fresh strawberries after
308 the different decontamination methodologies tested during 2-min exposure (Nicolau-Lapeña et
309 al., 2020; Ortiz-Solà et al., 2020).

310 **3.2. Foodborne pathogenic bacteria survival after cryogenic freezing process of strawberries**
311 **during shelf-life**

312 Freezing curves and phases of strawberry samples inoculated with *L. monocytogenes, S. enterica*
313 and murine norovirus (MNV-1) frozen at -70 ˚C in the cryogenic freezing cabinet are represented
314 in Fig. 3. The process observed using cryogenic technology has been the typical freezing process
315 used to freeze food at an extremely fast rate in the food industry (Comandini et al., 2013).

316 Cryogenic freezing process, evaluated after 3 days of storage at -25 °C, resulted in a sharp
317 reduction of *S. enterica* (Fig. 2A) regardless of the disinfection treatment carried out before
318 freezing, resulting in a final population of < 1.3 log CFU/g in all treatments ($P < 0.05$). Therefore,
319 the inactivation achieved during the frozen storage was sometimes rather substantial compared to
320 the inactivation caused the disinfection treatments used. In fact, reductions > 4 log units were
321 attributed to the freezing process for *S. enterica* in the untreated samples. On the contrary,
322 cryogenic freezing did not affect in a such way the viability of *L. monocytogenes* (Fig. 2B), with
323 reductions of 2 log approximately for all treatments, only with remaining populations about < 1.0
324 log on the surface of frozen strawberries treated with washing procedures.

325 For both pathogens, frozen storage at − 25 °C showed a slower but steady decline of bacterial
326 counts during the following 90 days. This survival behaviour of *L. monocytogenes* and *S. enterica*
327 on frozen strawberries was similar to previous investigations, which reported that a freezing step
328 had a greatest impact on *L. monocytogenes* and *E. coli* O157:H7 within the first 24 h of storage
329 at -20 °C, when populations in strawberry samples without added sucrose decreased by almost 1
330 log cycle. However, the short-term (30-day) survival of both pathogens was generally constant
331 on frozen strawberries (Flessa et al., 2005; Knudsen et al., 2001). On the other hand, the tailing
332 effect observed in the survival curve could be due to differences in resistance to acid/frozen
333 storage among the strains included in the 5-strain cocktail (Huang et al., 2013). It was previously
334 demonstrated that antimicrobial washing (chorine, chlorine dioxide and lactic acid) combined
335 with freezing step significantly reduced levels of *E. coli* O157:H7, *Salmonella* Typhimurium, and
336 *L. monocytogenes* in blueberries compared with what washing alone was capable of achieving in
337 maximum log reduction. In this study, wash treatments alone resulted maximum log reductions
338 from 0.4 to 2.0, while additional freezing step increased this to a range from 1.7 to 4.4 log
339 (Tadepalli et al., 2018). Similarly, Bridges et al. (2019) evaluated the efficacy of different
340 antibacterial washes coupled with frozen storage against foodborne pathogens on blueberries,
341 which were treated with sodium hypochlorite (NaClO, 200 ppm), chlorine dioxide (15 ppm),
342 ozone (3 and 5 ppm), or lactic acid (2 %) for short exposure times (10 s, 1 min, or 3 min) with an
343 additional freezing hurdle at –12 °C. They found that wash treatments alone resulted in maximum
344 log reductions from 1.0 to 2.8, while the additional freezing step increased this to a range from
345 3.7 to 6.6 after 1 week of storage. The greatest reduction of *L. monocytogenes* (6.6 log) and
346 *Salmonella* Typhimurium (5.3 log) was observed after freezing combined with 3 min of exposure
347 to 2 % lactic acid or 200 ppm of NaClO, respectively (Bridges et al. 2019).

348 After 90 days at frozen storage, *L. monocytogenes* and *S. enterica* were not detected on the
349 samples treated with water-assisted methodologies (WUVC, WUVC+PA and NaClO treatments).
350 However, the remaining population of *L. monocytogenes* was 2.8 ± 0.6 and 3.3 ± 0.8 log CFU/g
351 when the samples were treated with non-immersed DUVC treatment and untreated strawberries,

352 respectively. *S. enterica* was detectable by enrichment with 1 out of 3 samples being positive (<
353 5 CFU/g) after 90 days in the samples treated with DUVC. The results with bacteria on the surface
354 of the fruit yielded further decreases in *S. enterica* populations on untreated samples (CK)
355 throughout the experimental time, with reductions ca. 4-log compared to the initial level.
356 Nonetheless, *L. monocytogenes* population was maintained on CK samples after 180 days (1.8 ±
357 1.6 log CFU/g). These results showed that *L. monocytogenes* could survive on the surface of the
358 frozen fruit, although the populations declined markedly. Indeed, frozen foods do not support *L.*
359 *monocytogenes* growth while kept at freezing temperatures, but can survive for extended periods
360 on other food matrix (*e.g.,* for 120 days at - 18°C on whole and cut cucumbers (Bardsley et al.,
361 2019) or at least 28 days at -20°C on whole and cut strawberries (Flessa et al., 2005) particularly
362 in low acid food, including vegetables (Palumbo and Williams, 1991). Even if some cell injury
363 may occur, the reduction of the pathogen during the frozen storage of low acid fruits and
364 vegetables is very limited (ca. 1 $\log_{10}$ in 100 days) (Pappelbaum et al., 2008).

365 After one-year of frozen storage, both pathogens were completely inactivated. Even starting for
366 populations that are rather unrealistic, the use of WUVC and WUVC+PA before freezing
367 decreased the population of *L. monocytogenes* below 100 CFU/g (that allowed by EU Regulation
368 N° 2073/2005 and further amendments) after 3 days of storage.

369 **3.3. Effect of the sanitizing treatments on the infectivity of MNV-1 on strawberries after**
370 **sanitation procedures and cryogenic freezing step**

371 The initial virus titer of artificially inoculated strawberries was 3.4 ± 0.1 log $TCID_{50}$/mL. After 2-
372 min of disinfection treatments, the infectivity of MNV-1 was significantly lower compared with
373 that obtained with the untreated samples (CK) (Figure 4). Likewise pathogenic bacteria, washing
374 procedures are needed for the effective inactivation of MNV-1 infectivity, since DUVC treatment
375 proved to be the lowest effective technology. Reductions obtained with the water-assisted
376 sanitization treatments ranged between 1.8-1.9 log $TCID_{50}$/mL, whereas those obtained with the
377 DUVC methodology did not exceed 1.5 log $TCID_{50}$/mL ($P < 0.05$).

378 The infectivity of MNV-1 after cryogenic freezing process remained in frozen storage
379 strawberries (-25 °C) after 90 days, with no significant differences from the titer obtained after
380 disinfection step (Data not shown). In fact, Butot et al. (2008) observed that HAV and rotavirus
381 remained unaffected in unwashed fresh strawberries, raspberries, and blueberries, despite 90 days
382 in frozen storage. Baert et al., (2008) reported that no reduction of MNV-1 on spinach and spring
383 onions over 180 days of frozen storage. Unlike bacterial pathogens, maintaining the cold chain is
384 not a mitigation strategy for viral pathogens on fresh produce, as persistence of enteric viruses is
385 higher at low temperatures and inactivation rates generally increase with the increasing
386 temperatures (Li et al., 2015). It is not surprising that MNV-1 used in the present study was little
387 affected by freezing, as the results of our study corroborate data from documented outbreaks
388 involving HAV and human norovirus and linked with frozen berries (Cotterelle et al. 2005;
389 Falkenhorst et al. 2005; Hjertqvist et al. 2006; Hutin et al. 1999; Korsager et al. 2005). Indeed,
390 MNV-1 survived even the harsh conditions of cryogenic freezing with low pH of fruit. It has been
391 demonstrated that MNV-1 had tolerance to a low pH (pH 2 for 1 h; Li et al., 2013; Verhaelen et
392 al., 2013).

393 After 180 days, the infectivity of MNV-1 on untreated strawberries (CK) decreased, but remained
394 statistically higher compared with the frozen strawberries treated with the studied technologies
395 that were equally effective among them ($P > 0.05$). Results obtained with norovirus, showed again
396 that the disinfection step is needed, since infectivity of MNV-1 decreased after 360 days by > 1.6
397 log for both untreated and disinfected samples, with a remaining infectivity of ca. 1.5 and 0.1 log
398 $TCID_{50}$/mL, respectively. However, even low infectivity of MNV-1 was found on strawberries
399 after 360 days of storage, some investigations suggest that only 18 to 1,000 virus particles of
400 human norovirus are necessary to cause the disease (Teunis et al., 2008).

401 The results of the present study reported that WUVC and WUVC+PA treatments were equally
402 effective than 200 ppm of NaClO sanitization during experimental time ($P > 0.05$). It is well
403 known that biocides that have activity against both enveloped and non-enveloped viruses include
404 chlorine releasing agents, peracids and ozone. Their effectiveness depends on the nature of the

405 virus, the surface carrier, the presence of interfering substances such as organic soil or hard water
406 salts, and contact time (Vasickova et al., 2010). On the other hand, the mechanism involved in
407 antiviral activity of UV-C is probably the disruption of viral structure that ultimately degrades
408 viral proteins and RNA (Woo et al., 2019). Previous studies reported that subsequent UV-C
409 decontamination after other control strategies (*e.g.,* chlorine or peracetic acid) resulting in
410 synergistic benefits that could lead to increased UV-C induced viral genome damage (Rattanakul
411 et al., 2015). The effectiveness of UV-C light-based technologies is limited due to the shadow
412 effect. For this reason, the incorporation of water to the treatment enhanced the efficacy of this
413 innovative technology. Turbidity of the wash water used could affect UV-C penetration (Abadias
414 et al., 2021) and to extend exposure times for better UV-C efficacy may not be realistic and should
415 be further studied. Successful application of this combined technology relies on the light reaching
416 all the virus particles directly and, if the viruses are present in cracks, crevices or openings in the
417 surface of the food or surfaces, they could be inactivated with the chemical sanitizer present in
418 the water.

419 ## 4. Conclusions

420 Results from this study indicated that when the antimicrobial disinfection is combined with
421 freezing, injured bacterial cells that survived the washing step can be eliminated by exposure to
422 this second hurdle over time. The subsequent use of these frozen strawberries in retail food
423 operations or at home is particularly important when frozen berries are used in preparation of
424 smoothies, milkshakes and other foods not intended to go through a thermal process. Moreover,
425 we also showed that further research is warranted to address specific questions regarding the
426 effectiveness of disinfection step coupled with frozen storage against MNV-1, since human
427 norovirus is a highly stable virus that can survive from multiple days up to months on strawberry
428 surfaces at freezing conditions, making essential the introduction of a washing step in strawberry
429 processing. On the other hand, the amount of wastewater generated per mass unit of product
430 depends on the disinfection technique employed, so UV-C irradiation being capable of

431 disinfecting efficiently both the process water and the product, a higher ratio of recycling can be

432 achieved, with a lower impact on the environment.

433

434 **Acknowledgements**

435 The authors are grateful to the Spanish Government (Ministerio de Economía y Competitividad,
436 research project FRESAFE AGL2016-78086-R) and the CERCA Programme of 'Generalitat de
437 Catalunya' for its financial support. J. Ortiz-Solà thanks the University of Lleida for its PhD grant
438 (BOU186 - 243/2017 UdL). Authors are gratefully acknowledged to Carburos Metálicos for
439 supplying the Metal Carbide model Mini Batch CM-85/1090 equipment. We also thank Gloria
440 Sánchez and Susana Guix for teaching us the methodologies related to MNV-1 and RAW 264.7
441 assays and Marina Anguera for her technical support.

442

443 **Conflict of interests**

444 The authors declare no conflict of interests.

445

446 ## Authors statement

447 **Jordi Ortiz-Solà:** Conceptualization, Methodology, Investigation and Writing - Original
448 Draft. **Inmaculada Viñas**: Validation and Writing- Reviewing and Editing**. Ingrid**
449 **Aguiló-Aguayo**: Investigation. ***Gloria Bobo****:* Investigation. **Maribel Abadias**:
450 Conceptualization, Methodology, Visualization, and Writing- Reviewing and Editing.

451

452

453

454 **References**

455 Abadias, M., Colás-Medà, P., Viñas, I., Bobo, G., Aguiló-Aguayo, I. 2021. Application of an
456 innovative water-assisted ultraviolet C light technology for the inactivation of
457 microorganisms in tomato processing industries. *Food Microbiol.*, 94, 103631.
458 https://doi.org/ 10.1016/j.fm.2020.103631

459 Abadias, M., Usall, J., Anguera, M., Solsona, C. & Viñas, I. 2008. Microbiological quality of
460 fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int.*
461 *J. Food Microbiol.* 123(1–2), 121–129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013

462 Baert, L., Wobus, C.E., Van Coillie, E., Thackray, L.B., Debevere, J. & Uyttendaele, M. 2008.
463 Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time
464 reverse transcription- PCR before and after heat exposure. *Appl. Environ. Microbiol.*
465 74:543- 546. https://doi.org/10.1128/AEM.01039-07

466 Baert, L., Mattison, K., Loisy-Hamon, F., Harlow, J., Martyres, A., Lebeau, B. & Uyttendaele,
467 M. 2011. Review: Norovirus prevalence in Belgian, Canadian and French fresh produce: A
468 threat to human health?. *Int. J. Food Microbiol.*, *151*(3), 261–269.
469 https://doi.org/10.1016/j.ijfoodmicro.2011.09.013

470 Baicu, Adina & Popa, Mona. 2017. Trends in prolonging the post-harvest life of strawberries – a
471 review. In Annals of the University Dunarea de Jos of Galati, Fascicle VI: Food Technology;
472 Galati University Press: Galati, Romania, 2018;Volume 42, pp. 9–16.

473 Banach, K., Sampers, I., Van Haute, S., van der, H.J., F.K, 2015. Effect of disinfectants on
474 preventing the cross-contamination of pathogens in fresh produce washing water. *Int. J.*
475 *Environ. Res. Public Health,* 12, 8658–8677. https://doi.org/10.3390/ ijerph120808658.

476 Bardsley ,C.A., Truitt, L.N., Pfuntner, R.C., Danyluk, M.D., Rideout, S.L. & Strawn, L.K. 2019.
477 Growth and survival of *Listeria monocytogenes* and *Salmonella* on whole and sliced
478 cucumbers. *J Food Prot.,* 82, 301–309. https://doi.org/10.4315/0362-028X.JFP-18-341

479 Battino, M., Forbes-Hernandez, T.Y., Gasparrini, M., Afrin, S., Mezzetti, B. & Giampieri, F.
480 2017. The effects of strawberry bioactive compounds on human health. *Acta Hortic.*, (1156),
481 355–362. https://doi.org/10.17660/actahortic.2017.1156.54

482 Bernard, H., Faber, M., Wilking, H., Haller, S., Höhle, M., Schielke, A., Ducomble, T., Siffczyk,
483 C., Merbecks, S., Fricke, G., Hamouda, O., Stark, K., & Werber, D. 2014. Large multistate
484 outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012.
485 *Euro surveill.*, *19*(8). https://doi.org/10.2807/1560-7917.ES2014.19.8.20719

486 Bozkurt, H., Phan-Thien, K., Van Ogtrop, F., Bell, T., Mcconchie, R. 2020. Outbreaks,
487 occurrence, and control of norovirus and hepatitis a virus contamination in berries: A
488 review. *Crit. Re. Food sci,* 1-22. https://doi.org/10.1080/10408398.2020.1719383

489 Bridges, D. F., Tadepalli, S., Anderson, R., Zhang, R., & Wu, V. C. H. 2019. Reduction of *Listeria*
490 *monocytogenes* and *Salmonella* Typhimurium on blueberries through brief exposure to
491 antimicrobial solutions coupled with freezing. *J. Food Prot.*, 82(6), 926–
492 930. https://doi.org/10.4315/0362-028x.jfp-18-433

493 Butot, S., Cantergiani, F., Moser, M., Jean, J., Lima, A., Michot, L., Putallaz, T., & Zuber, S.
494 2018. UV-C inactivation of foodborne bacterial and viral pathogens and surrogates on fresh
495 and frozen berries. *Int. J. Food Microbiol*. 275, 8–16.
496 https://doi.org/10.1016/j.ijfoodmicro.2018.03.016

497 Butot, S., Putallaz, T., & Sanchez, G. 2008. Effects of sanitation, freezing and frozen storage on
498 enteric viruses in berries and herbs. *Int. J. Food Microbiol*., 126(1-2), 30–
499 35. https://doi.org/10.1016/j.ijfoodmicro.2008.04.033

500 CBI, Ministry of Foreign Affairs. 2019 [Online]. Available at:
501 https://www.cbi.eu/node/2764/pdf/. Accession date: 25/08/2020.

502 Comandini, P., Blanda, G., Soto-Caballero, M. C., Sala, V., Tylewicz, U., Mujica-Paz, H.,
503 Valdez-Fragoso, A. and Gallina Toschi, T. 2013. Effects of power ultrasound on immersion
504 freezing parameters of potatoes. *Innov. Food Sci. Emerg. Technol*., 18, 120–
505 125. https://doi.org/10.1016/j.ifset.2013.01.009

506 Cotterelle, B., C. Drougard, J. Rolland, M. Becamel, M. Boudon, S. Pinede, O. Traore, K. Balay,
507 P. Pothier, and E. Espie. 2005. Outbreak of norovirus infection associated with the
508 consumption of frozen raspberries, France, March 2005. *Euro Surveill.* 10 (4):E050428

509 Delgado, A. E.; Rubiolo, A. C. 2005. Microstructural changes in strawberry after freezing and
510 thawing processes. Lebensmittel Wissenschaft und-Technologie, v.38, p.135-142.

511 Dira. 2016. Frozen strawberries from Morocco: historic data and outlook 2016 [Online].
512 Available at: https://www.dirafro0073t.com/en/news-2/detail/frozen-strawberries-from-
513 moroccohistoric-data-outlook-2016. Accession date: 25/08/2020.

514 EFSA, Panel on Biological Hazards, 2014. Scientific opinion on the risk posed by patho-gens in
515 food of non-animal origin. Part 2 (*Salmonella* and Norovirus in berries). EFSAJ. 12,
516 3706.https://doi.org/10.2903/j.efsa.2014.3706.

517 Escudero, B. I., Rawsthorne, H., Gensel, C., & Jaykus, L. A. 2012. Persistence and Transferability
518 of Noroviruses on and between Common Surfaces and Foods. *J. Food Protect.,* 75(5),

519 927–935. https://doi.org/10.4315/0362-028x.jfp-11-460

520 Falkenhorst, G., L. Krusell, M. Lisby, S. B. Madsen, B. Bottiger, and K. Molbak. 2005. Imported
521 frozen raspberries cause a series of norovirus outbreaks in Denmark, 2005. *Euro Surveill.,*
522 10 (9):E050922

523 FAO (n.d.) United Nations Organization for Agriculture and Food. Department of agriculture and
524 consumer protection. More fruits and vegetables

525 Fennema, O. R., Powrie, W. D., & Marth, E. H. 1973. Lowtemperature preservation of foods and
526 living matter (p. 598). New York, USA: Marcel Dekker.

527 Flessa, S., Lusk, D. M., & Harris, L. J. 2005. Survival of *Listeria monocytogenes* on fresh and
528 frozen strawberries. *Int. J. Food Microbiol*. 101(3), 255–262.
529 https://doi.org/10.1016/j.ijfoodmicro.2004.11.010

530 Fruitlogistica: European statistics handbook, Berlin, Germany, 2020. 2020. [Online]. Available
531 at:
532 https://www.fruitlogistica.com/media/fl/fl_dl_all/auf_einen_blick/European_Statistics_Ha
533 ndbook_2020.pdf. Accession date: 25/08/2020

534 Fukuma, Y., Yamane, A., Itoh, T., Tsukamasa, Y., Ando, M., 2012. Application of supercooling
535 to long-term storage of fish meat. Fish. Sci. 78 (2), 451–461.

536 Giampieri, F., Forbes-Hernandez, T. Y., Gasparrini, M., Alvarez-Suarez, J. M., Afrin, S.,
537 Bompadre, S., Battino, M. 2015. Strawberry as a health promoter: an evidence based review.
538 *Food Funct*., 6(5), 1386–1398. https://doi.org/10.1039/c5fo00147a

539 Goodburn, C., and C. A. Wallace. 2013. The microbiological efficacy of decontamination
540 methodologies for fresh produce: a review. *Food Control* 32:418–427

541 Haffner, K. 2002. Postharvest quality and processing of strawberries. *Acta Hortic*. 567, 715-722.
542 https://doi.org/10.17660/ActaHortic.2002.567.157

543 Han, Y., R. H. Linton, and P. E. Nelson. 2004. Effects of recovery, plating, and inoculation
544 methods on quantification of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from
545 strawberries. *J. Food Prot*. 67 (11): 2436–2442. https://doi.org/10.4315/0362-028x-
546 67.11.2436

547 Hjertqvist, M., Johansson, A., Svensson, N., Abom, P.E., Magnusson, C., Olsson, M., Hedlund,
548 K.O. & Andersson, Y. 2006. Four outbreaks of norovirus gastroenteritis after consuming
549 raspberries, Sweden, June-August 2006. *Euro Surveill.* 11:E060907.1.

550 Huang, Y., Ye, M., Chen, H. 2013. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp.

551 in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage.
552 *Int. J Food Microbiol*., 160(3), 337–343. https://doi.org/10.1016/j.ijfoodmicro.2012.11.008

553 Hutin, Y.J.F., Pool, V., Cramer, E.H., Nainan, O.V., Weth, J., Williams, I.T., Goldstein, S.T.,
554 Gensheimer, K.F. Bell, B.P. & Shapiro, C.N. 1999. A multistate, foodborne outbreak of
555 hepatitis A. National Hepatitis A investigation team. *N. Engl. J. Med.,* 340 (8):595–602.,
556 https://doi.org/10.1056/NEJM199902253400802.

557 Janowicz, M., Lenart, A. & Idzikowska, W. 2007. Sorption properties of osmotically dehydrated
558 and freeze-dried strawberries. *Pol. J. Food Nutr. Sci.,* 57 (1), 69-76.

559 Knudsen, D.M., Yamamoto, S.A. & Harris, L.J. 2001. Survival of *Salmonella* spp. and
560 *Escherichia coli* O157:H7 on fresh and frozen strawberries. *J. Food Prot*., 64(10), 1483–
561 1488. https://doi.org/10.4315/0362-028X-64.10.1483

562 Korsager, B., Hede, S., Boggild, H., Bottiger, B.E. & Molbak, K. 2005. Two outbreaks of
563 norovirus infections associated with the consumption of imported frozen raspberries,
564 Denmark, May-June 2005. *Euro Surveill*., 10 (6):E050623.

565 Le Guyader, F.S., Mittelholzer, C., Haugarreau, L., Hedlund, K.O., Alsterlund, R., Pommepuy,
566 M. & Svensson, L. 2004. Detection of noroviruses in raspberries associated with a
567 gastroenteritis outbreak. *Int. J. Food Microbiol*., 97(2), 179–
568 186. https://doi.org/10.1016/j.ijfoodmicro.2004.04.018

569 Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *Int. J. Food Microbiol*,
570 55(1-3), pp. 181–186.

571 Li, D., De Keuckelaere, A. & Uyttendaele, M. 2015. Fate of foodborne viruses in the “farm to
572 fork” chain of fresh produce. Comprehensive reviews in food science and food safety, 14
573 (6), 755–70. https://doi.org/10.1111/1541-4337.12163.

574 Mäde, D., Trübner, K., Neubert, E., Höhne, M., & Johne, R. 2013. Detection and typing of
575 norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in
576 Germany. *Food Environ. Virol.*, *5*(3), 162–168. https://doi.org/10.1007/s12560-013-9118-0

577 Maunula, L., Roivainen, M., Keranen, M., Makela, S., Soderberg, K., Summa, M., von Bonsdorff,
578 Lappalainen, M., Korhonen, T., Kuusi, M. & Niskanen, T. 2009. Detection of human
579 norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks. *Euro Surveill*.
580 14:pii=19435.

581 Min, Z., Chunli, L., Yanjun, H., Qian, T., Haiou, W. 2001. Preservation of fresh grapes at ice-
582 temperature-high-humidity. *Int. Agrophys.* 15 (2), 139–143.

583 Nicolau-Lapeña, I., Abadias, M., Viñas, I., Bobo, G., Lafarga, T., Ribas-Agustí, A. & Aguayo, I.
584 A. 2020. Water UV-C treatment alone or in combination with peracetic acid: A technology
585 to maintain safety and quality of strawberries. *Int. J. of Food Microbiol*., 335, 108887.
586 http://doi.org/10.1016/j.ijfoodmicro.2020.108887

587 Ortiz-Solà, J., Abadias, M., Colás-Medà, P., Anguera, M. & Viñas, I. 2021. Inactivation of
588 *Salmonella enterica, Listeria monocytogenes* and murine norovirus (MNV-1) on fresh
589 strawberries by conventional and water-assisted ultraviolet light (UV-C). *Postharvest Biol.*
590 *Technol.*. 174, 111447. https://doi.org/10.1016/j.postharvbio.2020.111447

591 Ortiz-Solà, J., Abadias, M., Colás-Medà, P., Sánchez, G., Bobo, G. & Viñas, I. 2020. Evaluation
592 of a sanitizing washing step with different chemical disinfectants for the strawberry
593 processing industry. *Int. J. Food Microbiol*. 334, 108810.
594 https://doi.org/10.1016/j.ijfoodmicro.2020.108810

595 Palumbo, S.A. & Williams A.C. 1991. Resistance of *Listeria monocytogenes* to freezing in foods.
596 *Food Microbiol.*, 8, 63–68.

597 Pappelbaum, K., Grif, K., Heller, I., Wurzner, R., Hein, I., Ellerbroek, L. & Wagner, M. 2008.
598 Monitoring hygiene on- and at-line is critical for controlling *Listeria monocytogenes* during
599 produce processing. *J Food Prot*., 71, 735–741. http://doi.org/10.4315/0362-028x-71.4.735

600 Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J. &
601 Calderon, R.L. 2008. Norwalk virus: How infectious is it?. , 80(8), 1468–
602 1476. https://doi.org/10.1002/jmv.21237

603 Pintó, R.M., Diez, J.M. & Bosch, A. 1994. Use of the colonic carcinoma cell line CaCo-2 for in
604 vivo amplification and detection of enteric viruses. *J Med. Virol*. 44, 310-315.
605 https://doi.org/10.1002/jmv.1890440317

606 RASFF. 2020. 22 notifications of enteric viruses on red fruits and berries, including strawberries,
607 redcurrants, blueberries, blackberries, cherries, blackcurrants and raspberries (from
608 1/1/2018 to 31/12/20). https://webgate.ec.europa.eu/rasff-
609 window/portal/?event=searchResultList/. Accessed 27/01/21

610 Rattanakul, S., Oguma, K. & Takizawa, S. 2015. Sequential and simultaneous applications of UV
611 and chlorine for adenovirus inactivation. *Food Environ. Virol.* 7.
612 https://doi.org/10.1007/s12560-015-9202-8.

613 Rizzo, C., Alfonsi, V., Bruni, R., Busani, L. & Ciccaglione, A. 2013. Ongoing outbreak of
614 hepatitis A in Italy: preliminary report as of 31 May 2013. *Euro surveill*. 2013:18

615 Samadi, S., Ghasemnezhad, A. & Imani, J. 2017. Extending shelf life of strawberry using some

616 pre-storage treatments. *Acta Horticulturae*, 1156, 643-652.
617 https://doi.org/10.17660/ActaHortic.2017.1156.94

618 Sánchez, G., Aznar, R., Martínez, A., Rodrigo, D. 2011. Inactivation of human and murine
619 norovirus by high-pressure processing. *Foodborne Pathog. Dis*. 8(2), 249–253.
620 https://doi.org/10.1089/fpd.2010.0667

621 Sarvikivi, E., Roivainen, M., Maunula, L., Niskanen, T., Korhonen, T., Lappalainen, M. & Kuusi,
622 M. 2012. Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiol.*
623 *Infect., 140*(2), 260–267. https://doi.org/10.1017/S0950268811000379.

624 Severi, E., Verhoef, L., Thornton, L., Guzman-Herrador, B.R., Faber, M., Sundqvist, L.,
625 Rimhanen-Finne, R., Roque-Afonso, A.M., Ngui, S.L., Allerberger, F., Baumann-Popczyk,
626 A., Muller, L., Parmakova, K., Alfonsi, V., Tavoschi, L., Vennema, H., Fitzgerald, M.,
627 Myrmel, M., Gertler, M., Ederth, J., Kontio, M., Vanbockstael, C., Mandal, S., Sadkowska-
628 Todys, M., Tosti, M.E., Schimmer, B., Gorman, J., Stene-Johansen, K., Wenzel, J.J., Jones,
629 G., Balogun, K., Ciccaglione, A.R., O' Connor, L., Vold, L., Takkinen, J. & Rizzo, C. 2015.
630 Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with
631 consumption of frozen berries, 2013 to 2014. *Euro Surveill.* 20:21192.

632 Stonehouse, G.G. & Evans, J.A. 2015. The use of supercooling for fresh foods: a review. *J. Food*
633 *Eng.* 148, 74–79. https://doi.org/10.1016/j.jfoodeng.2014.08.007

634 Sun, D., Pan, T.T., Pu, H.B., Zhu, Z.W. & Han, Z. 2019. Multi-temperature-region ice-
635 temperature fresh keeping storehouse and fresh keeping method for bergamot pears, 16/324,
636 586. U.S. Patent Application No.

637 Tadepalli, S., Bridges, D.F., Driver, R. & Wu, V.C.H. 2018. Effectiveness of different
638 antimicrobial washes combined with freezing against *Escherichia coli* O157:H7,
639 *Salmonella* Typhimurium, and *Listeria monocytogenes* inoculated on blueberries. *Food*
640 *Microbiol*., 74, 34–39. https://doi.org/10.1016/j.fm.2018.02.018.

641 Tavoschi, L., Severi, L., Niskanen, T., Boelaert, F., Rizzi, V., Liebana, E., Gomes-Dias, J.,
642 Nichols, G., Takkinen, G. & Coulombier, D. 2015. Food-borne diseases associated with
643 frozen berries consumption: a historical perspective, European Union, 1983 to 2013. *Euro*
644 *surveill.,* 20 (29), 1-11. https://doi.org/10.2807/1560-7917.ES2015.20.29.21193

645 Tournas, V.H., Heeres, J. & Burgess, L. 2006. Moulds and yeasts in fruit salads and fruit juices.
646 *Food Microbiol.* 23, 684–688. https://doi.org/10.1016/j.fm.2006.01.003

647 Velickova, E., Tylewicz, U., Dalla Rosa, M., Winkelhausen, E., Kuzmanova, S. & Romani, S.
648 2018. Effect of pulsed electric field coupled with vacuum infusion on quality parameters of

649 frozen/thawed strawberries. *J. Food Eng.*, 233, 57–
650 64. https://doi.org/10.1016/j.jfoodeng.2018.03.030

651 Verhaelen, K., Bouwknegt, M., Carratalà, A., Lodder-Verschoor, F., Diez-Valcarce, M.,
652 Rodríguez-Lázaro, D., Rutjes, S. A. 2013. Virus transfer proportions between gloved
653 fingertips, soft berries, and lettuce, and associated health risks. *Int. J. Food Microbiol*,
654 166(3), 419–425. doi:10.1016/j.ijfoodmicro.2013.07.025

655 Wang, S.Y. 2014. Antioxidants and health benefits of strawberries. *Acta Hortic.*, (1049), 49–
656 62. https://doi.org/10.17660/actahortic.2014.1049.1

657 Woo, H., Beck, S.E., Boczek, L.A., Carlson, K.M., Brinkman, N.E., Linden, K.G., Lawal, O.R.,
658 Hayes, S.L. & Ryu, H. 2019. Efficacy of inactivation of human enteroviruses by dual-
659 wavelength germicidal ultraviolet (UV-C) light emitting diodes (LEDs). *Water Res.*, 11(6),
660 1131–. https://doi.org/10.3390/w11061131

661 Wright, K.P. & Kader, A.A. 1997. Effect of controlled-atmosphere storage on the quality and
662 carotenoid content of sliced persimmons and peaches. *Postharv. Biol. Technol.*, 10, 89-97.
663 http://dx.doi.org/10.1016/S0925-5214(96)00062-2

664 Yu, K., Newman, M.C., Archbold, D.D & Hamilton-Kemp, T.R. 2001. Survival of *Escherichia*
665 *coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical
666 agents. *J. Food Prot*. 64 (9), 1334–1340. https://doi.org/10.4315/0362-028x-64.9.1334

667

668 **Table 1.-** Water parameters: pH, ORP, concentration of sanitizer and *L. monocytogenes* and *S.*
669 *enterica* population in washing water after 2 min exposure. Pathogenic bacteria population
670 values are the mean of the 4 repetitions ± standard deviation.

Treatment[1]	[ ] of free chlorine or PA (mg/L)	pH	ORP (mV)	*L. monocytogenes*	*S. enterica*
WUV-C	< 0.01	7.7 ± 0.6	201.5 ± 12.1	0.80 ± 0.59 (3/4)[2]	0.72 ± 0.52 (3/4)[2]
WUV-C+PA	40.7 ± 2.7	5.3 ± 0.2	465.0 ± 2.8	ND*	ND*
NaClO	184.0 ± 3.5	6.7 ± 0.1	879.5 ± 2.1	ND*	ND*

671 [1] WUV-C: water-assisted ultraviolet disinfection, WUV-C + PA: ultraviolet disinfection combined with
672 peracetic acid, NaClO: hypochlorite solution.
673 [2]: Number samples that were confirmed positive after enrichment in Dey-Engley / total samples analyzed
674 ND*: no-detected in the wash water
675

Figure 1. (A) Cryogenic freezing cabinet (Carburos Metálicos – Air Products Group model Batch freezer CM-85/1090) which is cooled by injecting liquid Nitrogen (N_2) from Carburos Metalicos (B). (C and D) Frozen strawberry samples after freezing process.

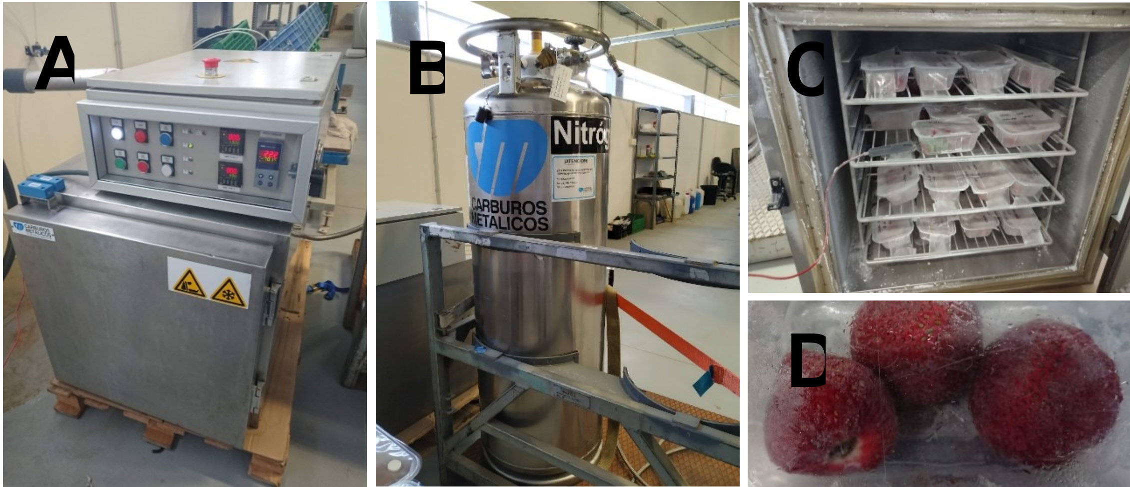
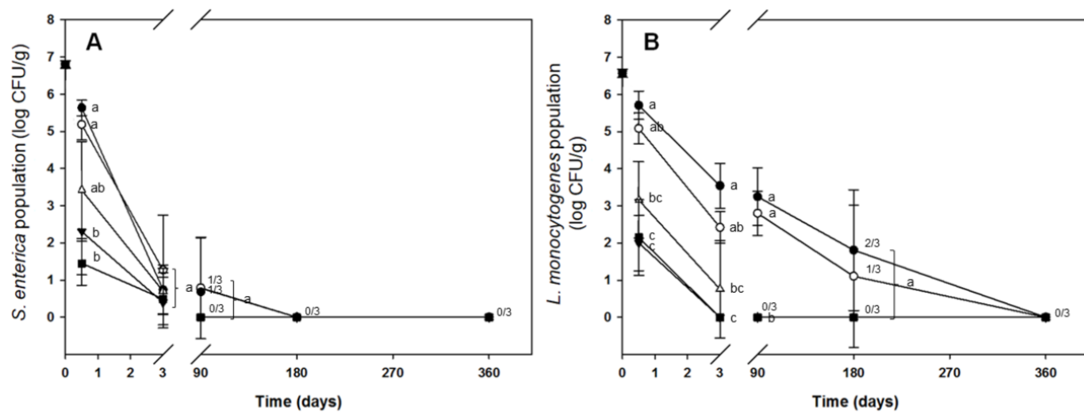
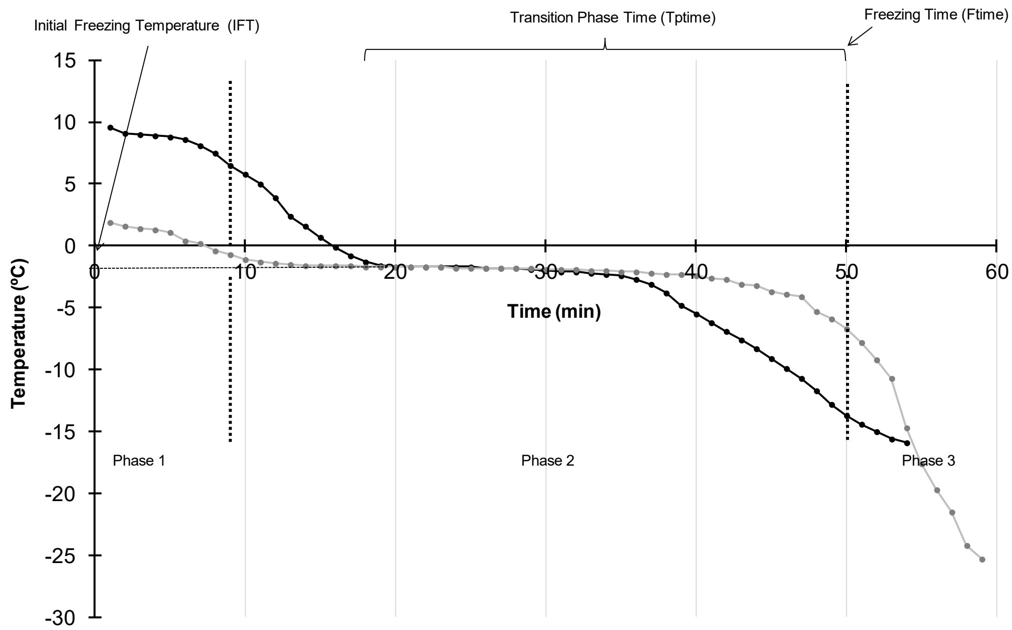


Figure 2. Population (log CFU/g) of *Salmonella enterica* (A) and *Listeria monocytogenes* (B) as a function of the applied disinfection treatments and storage time (n=3). CK: control samples (without washing) (•), DUVC: conventional dry ultraviolet disinfection (○), NaClO: hypochlorite solution (200ppm) (▾), WUV-C: water-assisted ultraviolet disinfection (▵), WUV-C + PA: water-assisted ultraviolet disinfection combined with peracetic acid (40 ppm) (■). For each represented time, different letters indicate significant differences among disinfection treatments according to the HSD Tukey post-hoc test ($p<0.05$). Numbers in fraction represent the number of samples testing positive after enrichment out of the total analyzed samples (3).



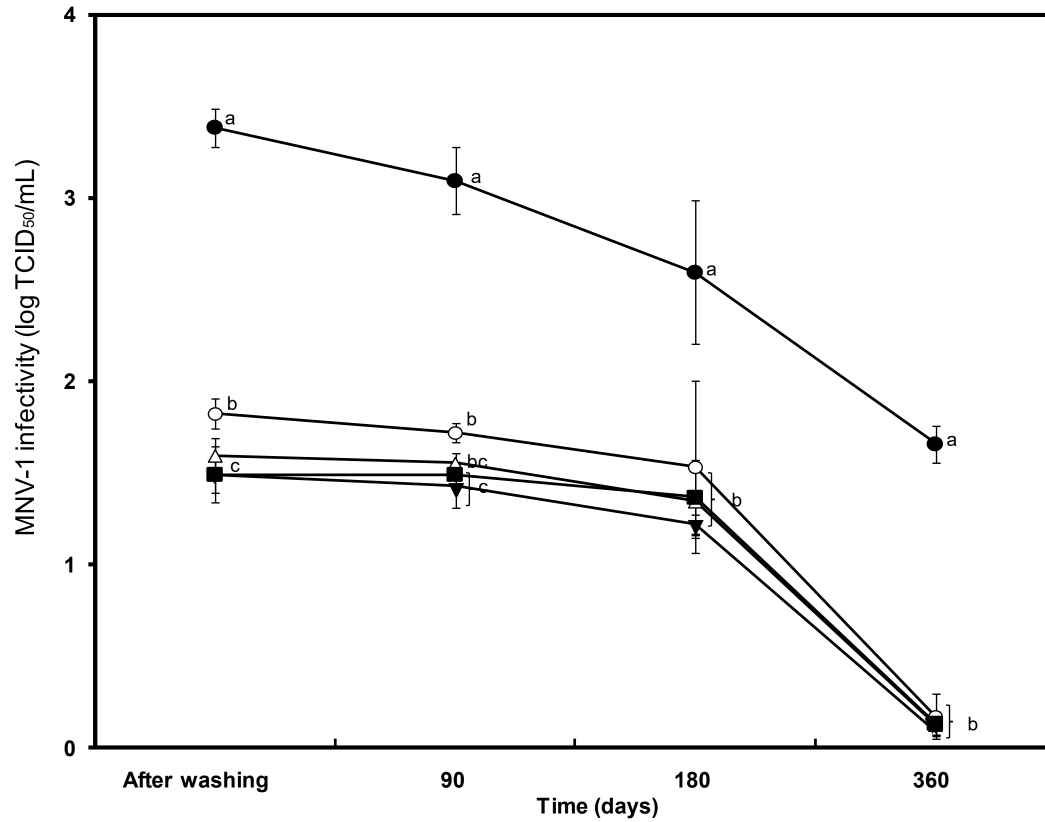
691 **Figure 3.** Freezing parameters and curves of strawberry samples inoculated with *Listeria*
692 *monocytogenes* and *Salmonella enterica* (black) and murine norovirus (MNV-1) (grey) frozen at
693 -70 ˚C in a cryogenic freezing cabinet comprising liquid nitrogen (N_2), from the brand Carburos
694 Metálicos – Air products Group model Batch freezer CM-85/1090.



695

696

697 **Figure 4.** Infectivity (log $TCID_{50}$/mL) of the MNV-1 in relation to the applied disinfection
698 treatments and different storage time (n=6). CK: control samples (without washing) (•), DUVC:
699 conventional dry ultraviolet disinfection (○), NaClO: hypochlorite solution (200ppm) (▾), WUV-
700 C: water-assisted ultraviolet disinfection (▵), WUV-C + PA: water-assisted ultraviolet
701 disinfection combined with peracetic acid (40 ppm) (■).



702

703

704