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Cyclic low dose UV-C treatments retain strawberry fruit quality more effectively than conventional pre-storage single high fluence applications

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Abstract

UV-C irradiation has been shown to reduce fruit decay and delay ripening. Based on an expected higher impact and applicability, UV irradiation treatments have been almost exclusively done before storage at relatively high doses. We evaluated the influence of the pattern of repeated short dose UV-C exposure on quality maintenance of strawberry fruit. Strawberries were subjected to the following treatments: S*ingle-step UV*: single 4 kJ m^{-2} irradiation prior to storage; *two-step UV*: two consecutive 2 kJ m^{-2} UV irradiations at harvest and after 4 days of storage and *multi-step UV*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 days of storage respectively. A non-irradiated group was left untreated. Samples were stored at 0 °C for 13 days. All UV-C treatments decreased decay, weight loss and softening. The quality retention was higher in fruit subjected *two-step* and *multi-step* UV-C. Multiple low dose UV exposure reduced calyx browning more efficiently. Repeated low UV-C dose decreased mold and yeast counts to a higher extent. *Multi-step* UV treated fruit showed higher alcohol insoluble residue. *Two-step* UV-C treated fruit showed the highest sensorial scores. Repeated low dose UV-C treatments are more effective in preventing strawberry fruit than conventional single high-fluence pre-storage irradiation.

Keywords: *Fragaria* x *ananassa* Duch; UV-illumination; ripening; *Botrytis cinerea*

1. INTRODUCTION

Strawberries are widely appreciated for their bright red color, unique aroma and texture and high antioxidant capacity (**Giné-Bordonaba and Terry 2016; Li et al. 2017**). However, continuous distribution of premium berries is challenging due to their high perishability (**Bower et al. 2003; Chen et al. 2016**). Though low temperature storage effectively reduce deterioration, even under proper temperature management strawberry shelf-life life rarely exceeds 7-10 days (**Erkan et al. 2008**). To date, no postharvest fungicides have been approved to control *Botrytis*

cinerea and other wet treatments such as washing are not recommended since they may increase decay susceptibility (**Mitcham 2016**). Firming agents such as calcium salts (**Aguayo et al. 2008**) which has been effectively shown to delay softening and fungal attack is mainly limited to the fresh-cut industry whereas strategies such as surface coating showing good results at lab scale (**Romanazzi et al. 2016**) have had difficulties to be up-scaled to commercial settings. Consequently, there is a relatively limited set of strategies to reduce postharvest losses in strawberry limited.

In recent years there have been great interests in the search for non-chemical dry methods that can prevent fruit deterioration (**Vicente and Lurie 2014**). Several research groups have started to evaluate UV-C treatments as a potential alternative to control spoilage (**Civello et al. 2006; Xu et al. 2017**). Strawberry pre-storage UV-C treatments at doses ranging from 0.2 to 4.2 kJ m^{-2} reduced decay (**Baka et al. 1999; Erkan et al. 2008; Li et al. 2014; Xu et al. 2017**). UV-C radiation has been shown to affect fungal metabolism (**Bintsis et al. 2000; Trivittayasil et al. 2016**). **Pan et al. (2004)** reported that UV-C radiation (4 kJ m^{-2}), reduced the rate of germination of *Botrytis* and *Rhizopus* conidia. Besides its direct effect on plant pathogens, UV-C radiation has been shown to modulate ripening-associated processes such as softening (**Stevens et al. 2004; Pan et al. 2004**) and to elicit the accumulation of antioxidants (**Erkan et al. 2008**) and phytoallexins (**D´hallewin et al. 1999**). **Pombo et al. (2011)** reported that UV-C irradiation may increase the expression of chitinases and β-1,3-glucanases. Early work by **Nigro et al. (2000)** also reported the induction phenylpropanoid regulatory enzymes such as phenylalanine ammonia lyase (PAL).

Several factors determining the efficacy of UV treatments have been studied; the maturity stage at which the fruit is irradiated influenced the outcome of UV treatments, with early applications having more dramatic effect delaying ripening (**Liu et al. 1993; Charles et al. 2002**). However, strawberries must be picked at complete maturity in order to attain full flavor, what narrows the window at which UV treatments could be applied. The UV radiation dose (fluence) applied also affects the benefits of UV-C treatments on fruit quality maintenance. This has been, by far the variable most extensively studied (**Civello et al. 2014**). **Cote et al.**

(2013) showed that for a given dose radiation intensity also affects the efficacy of UV-C treatments. Other factors such as the as the pattern of UV exposure have been barely studied. Even though pre-storage applications would be more practical than cyclic UV-C understanding the responses of fruit to different irradiation conditions is very important to better understand the physiological effects of postharvest photochemical treatments. No studies have been conducted to determine if small point applications throughout the storage period could improve quality retention relative to conventional single high fluence UV treatments. The aim of this work was to determine if repeated short applications throughout the storage period could improve quality retention relative to conventional single high fluence UV treatments

2 MATERIALS & METHODS

2.1 Plant material, treatments and storage conditions

Strawberry fruit (*Fragaria* x *ananassa* cv Camarosa) grown in La Plata, Argentina was harvested at commercial maturity and immediately transported to the laboratory. Fruit was put in polyethylene terephthalate (PET) trays, in groups of 10 and was located under an irradiation mobile bank (1.7 m x 0.8 m) consisting a closed cabinet containing on the upper side 12 UV-C lamps (UV-C peak emission at 254 nm, TUV G30T8, 30 W, Philips, Argentina) with a global maximum radiation intensity of 38 W m^{-2}. The fruit was rotated in order to irradiate two opposite sides. Fruit was irradiated at a distance of 30 cm. UV-C radiation dose was evaluated by using a digital UV-C radiometer (ElectroLite Miodel LC 300, USA) located in the central zone of the irradiation zone. The following treatments were applied:

i) *Single-step UV*: 4 kJ m^{-2} application before storage;

ii) T*wo-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage

iii) *Multi-step UV*: five 0.8 kJ m^{-2} applications after 0, 2, 4, 6 and 8 d of storage.

One set of non-irradiated fruit was used as a *Control*. Samples were covered with a perforated plastic lid and stored 0, 10 or 13 days at 0 ºC. For those treatments requiring UV exposure during the storage period the bank was used directly into the storage area to avoid oscillations in fruit temperature. Samples were immediately analyzed after sampling or otherwise frozen in liquid N_2 and stored at -80 °C until analysis. Four trays containing 10 fruit each were used for every treatments and storage time. The whole experiment was repeated three times.

2.2. Respiration rate

Samples were taken and held at 20 °C until thermal equilibration. Ten fruits were placed in a 1.5 L glass jar which was hermetically sealed. An IR sensor (Alnor, USA) was used to determine the change in CO_2 in the headspace during a 20 min period. The respiration rate was calculated by determining the mass of CO_2 produced per kg of fruit in an hour. Three measurements were done for each treatment and sampling date.

2.3. Weight loss

Individual fruits were weighed at the beginning of the storage period and after 10 or 13 d at 0 °C. Weight loss was calculated as: $WL = 100\times (W_i - W_f)/Wi$, being W_i the initial sample weight and W_f the final weight. Results were expressed in percentage.

2.4. Decay

The percentage of fruit showing incipient symptoms of decay (local tissue maceration) or excessive softening on each tray was recorded. Decay incidence was expressed as percentage of decayed fruits. Four trays containing 10 fruit each were evaluated for each treatment and storage time.

125 **2.5. Color**

126 Fruit calyx and receptacle color was measured with a colorimeter (Model CR-400,
127 Minolta, Osaka, Japan) to obtain L*, a* and b* values. The hue angle was calculated as 180 -
128 tg^{-1} (b*/a*) and tg^{-1} (b*/a*) for fruit calyx and receptacle respectively. For fruit receptacle color
129 assessment two measurements were conducted on each fruit and averaged. Thirty fruits were
130 evaluated for each treatment and storage time and evaluated for both receptacle and calyx color.

131

132 **2.6. Firmness**

133 Fruit firmness was determined by uniaxial compression tests in a Texture Analyzer
134 (TA.XT2, Stable Micro Systems Texture Technologies, NY, USA) equipped with a 3 mm
135 diameter flat probe. Firmness was determined compressing the fruit tissue 4 mm in equatorial
136 zone at a rate of 1 mm s^{-1}. The maximum force during the test was recorded. Forty
137 measurements were done for each treatment and time analyzed.

138

139 **2.7. Isolation of cell wall material and determination of alcohol insoluble residue**

140 Cell wall polysaccharides were isolated as previously described (**Vicente et al. 2007**).
141 Fruit samples were immediately placed in 95% (v/v) ethanol to limit the action of cell wall
142 modifying enzymes isolated with the tissue. Approximately 30 g of tissue (exocarp plus
143 mesocarp) for each developmental stage was homogenized in an UltraTurrax (IKA Werke Janke
144 & Kunkel GmbH & Co. KG, Staufen, Germany) with 75 mL of 95% ethanol and boiled for 45
145 min to ensure the inactivation of enzymes, thus preventing autolytic activity, and to extract low
146 molecular weight solutes. The insoluble material was filtered through Miracloth (Calbiochem,
147 EMD Biosciences, Inc., San Diego, CA) and sequentially washed with 150 mL of boiling
148 ethanol, 150 mL of chloroform/ methanol (1:1 v/v), and 150 mL of acetone, yielding the crude

149 cell wall extract (alcohol insoluble residue, AIR). The AIR was dried overnight at 37 °C and
150 weighed. Results were expressed as milligrams of AIR per gram of fresh fruit.

151

152 **2.8. Titratable acidity**

153 Fruit pulp was frozen in liquid nitrogen, ground in a mill and 10 g of the resulting
154 powder were added to 100 mL of water. Samples were titrated with 0.1 mol L^{-1} NaOH until pH
155 8.2 (**AOAC 1980**). Results were expressed as H^+ mmol per kg^{-1} on a fresh weight basis. Three
156 measurements were done for each treatment and storage time.

157

158 **2.9. Sugars**

159 Approximately 50 g of fruit tissue were ground in a mill and 1 g of the resulting powder
160 was homogenized with 10 mL of ethanol and vortexed for 1 min. The mixture was centrifuged
161 at 9,000 x *g* for 10 min at 4 ºC; the supernatant was recovered and filtered through 0.2 mm RC
162 membrane (Cole-Parmer, USA) and brought to 50 mL with deionized water. A high-
163 performance liquid chromatograph (HPLC, Waters 1525 Binary HPLC Pump) was used,
164 equipped with a refractive index detector (Waters, IR 2414) and a Hypersil Gold Amino column
165 (4.6 x 250 mm, 5 mm, Thermo Sci., USA). Samples were run with an isocratic flow rate of 1.0
166 mL min^{-1} of acetonitrile: water (70: 30). Three extracts were analyzed per treatment and storage
167 times and measurements were done in duplicate. Results were expressed as g of sugar per kg.

168

169 **2.10. Ascorbic acid**

170 Samples were frozen in liquid nitrogen, processed in a mill and approximately 1 g of the
171 obtained powder was homogenized with 5 mL of 2.5% m/v metaphosphoric acid. The mixture
172 was vortexed for 1 min and then centrifuged at 12,000 x *g* for 10 min at 4 ºC. The supernatant
173 was recovered and filtered through 0.45 µm (MSI Westboro, MA 01581, 100pk acetate plus)

membrane and ascorbic acid (AsA) determination was done by using a high-performance liquid chromatograph (HPLC, Waters 1525 Binary HPLC Pump), fitted with a photo diode array detector and a C18 column (4.6 x 150 mm, 5 mm, Waters Corp., USA). The mobile phase was 0.5% m/v metaphosphoric acid/acetonitrile (93/7) at an isocratic flow rate of 1.0 mL min^{1} and the wavelength for detection was 245 nm. For identification and quantitation a standard AsA solution was employed. Results were expressed as mg of AsA per kg. Two extracts per sample and storage time were obtained. All samples were run twice and averaged.

2.11. Microbiological counts

Approximately 50 g of fruit were put into two sterilized beakers containing 225 mL 0.1% w/v peptone. Samples were stirred for 15 min and from each beaker a series of decimal dilutions was prepared. One mL samples from different dilutions (10^{-2} to 10^{-5}) were poured in triplicate into the Yeasts and Molds culture medium (Petrifilm™ plates 6407, 3M, St. Paul, Minn., U.S.A.). Plates were incubated at 20 ºC. Results were expressed as log of viable colony forming units (CFUs) per g of fresh fruit.

2.12. Sensory evaluation

Fruit visual sensory evaluation was assessed by an acceptability test using a hedonic scale of 9 points. Panelist were simultaneously offered trays containing 10 whole strawberries having similar size and shape from control, *one step UV*, *two-step UV* and *multi-step UV*. The fruit was evaluated after 10 day of storage at 0 ºC. The panelist were asked to indicate their acceptability on a 9 point hedonic scale, being 1: unacceptable and 9: highly accepted. The evaluation considered those attributes that may be considered on an initial purchase decision (calyx color, receptacle color, freshness and overall acceptability). The panel consisted on 100 non-trained panelists with equal distribution of men and women and with an age range of 25-35 years.

200

201 **2.13. Statistical analysis**

202 Samples were analyzed by a ANOVA with the PC-SAS software package (SAS 198
203 Institute Inc., Cary, NC). The model assumptions of homogeneity of variance and normality
204 were tested by means of the Levene and Shapiro-Wilk tests, respectively. Treatment means were
205 compared using Tukey's studentized range test (*$*P < 0.05$*).

206

207 **3. RESULTS AND DISCUSSION**

208 **3.1 Weight loss, decay, microbial counts and phenolic compounds**

209 UV-C treatments reduced fruit weight loss during storage. After both 10 and 13 days all
210 UV treatments reduced dehydration regardless of the mode of application. Remarkably the least
211 water loss values were observed in the case of the fractionated UV treatments **(Figure 1**). *Two-*
212 *step UV-C* exposure had a similar effect on fruit weight loss than *multi-step* irradiation. Previous
213 work has showed that high intensity UV radiation treatments, applied before storage, can reduce
214 water loss in strawberry (**Cote et al. 2013**). Whether this effect resulted simply an improved
215 maintenance of fruit integrity or from changes in fruit surface characteristics is unknown. In
216 fresh-cut apple the lower water loss resulting from UV-C was associated with the formation of a
217 thin film on the product surface hindering water evaporation (**Manzocco et al. 2011**). This was
218 not evident at least by direct stereomicroscopic observations (data not shown). Other potential
219 effects induced by UV radiation that can affect the rate of water loss include changes in surface
220 wax deposition (**Charles et al. 2008**) or modifications in the degree stomata closure (**He et al.**
221 **2011**). Though these responses seem less likely given the low storage temperature, they could
222 not be discarded and further work aimed in understanding the mechanism by which *two-step*
223 and *multi-step UV-C* irradiation reduces fruit susceptibility to dehydration would be of interest.

After 10 days at 0 °C no differences in fruit decay were found between control and conventionally (*one-step*) UV-C treated strawberries. In contrast, the both *two-step* and *multi-step UV-C* treated fruit showed no decay (**Figures 2**). After 13 days at 0 °C a rapid increase in fruit decay was found in control strawberries. At this sampling date, fruit subjected to single pre-storage UV irradiation presented lower decay incidence than the corresponding control. This is coincident with previous work showing that single UV irradiation at doses ca. 4 kJ m^{-2} can be useful to control decay by *Botrytis cinerea* (**Pan et al. 2004; Cote et al. 2013**). Interestingly, also after long term storage *two-* and *multi-step* applications relying on repeated exposure at low radiation fluence were significantly more effective than single pre-storage irradiation to control decay.

We subsequently evaluated the viable count of molds and yeast (**Figure 3)**. The colonies counted represented mostly yeasts. At harvest 3.4 CFU g^{-1} were found. Immediately after the initial irradiation there was a significant decrease in yeast counts all the treatments. The greatest reduction was observed in one step treatments receiving the highest radiation dose at day 0 (4 kJ m^{-2}). Work by Mercier et al. has shown that direct inactivation of microorganisms by UV radiation is highly dependent on radiation dosage (**Mercier et al., 2001**). The fractionated *two-step* and *multi-step* treatments showed no differences in yeast counts reduction prior to storage. In this case, a significant but modest reduction (*ca*. 0.25 log cycles) was observed. The counts of control strawberries increased one log cycle during 10 days of storage. Fruit subjected to *one-step* UV irradiated also showed an increasing trend. Remarkably, the counts in fruit subjected to low fluence *two-* and *multi-step* UV-C showed no changes in yeast counts throughout the storage period, but rather a decrease. This shows that, for a similar total radiation dosage, repeated low dose UV-C exposure *in vivo* resulted in a better more effective reduction in yeasts CFU than one step irradiation. One plausible explanation is that the repeated irradiation, even with low partial doses, was sufficient to exert inhibitory effects on yeast viability and that several treatments was more detrimental (**Nhung et al. 2012; Sinha and Häder 2002**). Despite of the potential direct effect that repeat low dose UV exposure could exert on fruit pathogens we cannot exclude that fractionated UV could have induced defensive responses. Early work by

Ben-Yehoshua (1992) clearly showed that UV-C irradiation induced the accumulation of the phytoalexin scoparone in citrus flavedo. Subsequent studies in even in strawberry reported that UV-C irradiation also increased the activity of enzymes related to active responses such as chitinases and β-1,3 glucanases (**Pombo et al. 2011**) or associated with the biosynthesis of antimicrobial phenolics (**Nigro et al., 2000; Erkan et al. 2008**). For successful colonization, a pathogen must succeed over the fruit's defensive arsenal. This could be done even for a single microorganism by many different mechanisms depending on the prevailing physiological and environmental conditions. **Prusky et al., (2016)** recently suggested that carbon availability in the environment is a key factor triggering the production and secretion of ammonia and organic acids which could modulate the pH and result in completely different pathogenic responses. Then, it would be interesting to evaluate whether the pattern of exposure to UV-C radiation could affect carbon status within the apoplast and contribute to affect pathogen invasion.

3.2 Receptacle and calyx color, respiration, sugars, acidity and ascorbic acid

Fruit receptacle hue and lightness decreased indicating that ripening progressed even during storage at 0 °C (**Table 1**). The receptacle hue decreases during storage from 45º at the beginning to 30º at the end of storage period. No differences in hue values were recorded between control and UV irradiation fruit for any treatment schedule evaluated (**Table 1**). This is consistent with the results reported by **Pan et al. (2004)** who found subtle color changes in UV-C treated strawberries. The UV treatments induced a slight reduction in receptacle lightness (L*). However, this effect was very limited compared to the browning recorded during the 13-d storage period. At the last sampling date, the *two-step UV-C* treatments caused lower lightness loss than control (**Table 1**). Calyx hue angle dropped in all treatments from values *ca.* 130 at harvest to 120 at the last sampling date in association with chlorophyll degradation. In accordance with **Marquenie et al. (2002)** we did find some calyx browning and drying in UV-C treated strawberries. The reduction of calyx L* values was delayed in *two-* and *multi-step* UV treatments (**Table 1**).

279 Fruit respiration rate showed an increasing trend during storage in control and treated
280 fruit (**Table 2**). Though strawberry has a non-climacteric ripening pattern of respiration previous
281 works have reported that CO_2 production can increase especially after long term storage
282 (**Vicente et al., 2006**). This has been mainly related to prolonged stress conditions occurring in
283 the postharvest environment such as water and nutrient deprivation and pathogen challenges
284 that may result in fruit damage (**Li and Kader 1989**). After 13 d at 0 ºC, UV-C irradiated fruit
285 maintained lower respiration levels than the remaining treatments. This indicates that UV
286 irradiation reduced fruit deterioration and may be useful to maintain lower metabolic activity.
287 We further determined changes in acidity sugars and ascorbic acid to determine if these
288 components contributing to fruit taste and nutritional quality were affected by the UV-C
289 treatment schedule. Fruit acidity and ascorbic acid content did show no major changes during
290 storage and were not affected by any of the UV-C treatments evaluated. Glucose and fructose
291 represented 65% of total fruit sugars at harvest (**Table 2**). During storage, they showed an
292 increasing trend, with a concomitant decrease in sucrose likely probably resulting from
293 invertase action as reported by **Basson et al. (2010**). However, this trend was similar in control
294 in all the UV treatments evaluated. Overall this shows that UV-C treatments did not cause major
295 changes in soluble sugars acid or ascorbic acid metabolism at the whole fruit level.

296

297 **3.3 Firmness and cell wall material**

298 No significant differences were found in firmness prior to cold storage were found among
299 treatments. As expected the fruit soften markedly during storage. Though strawberries subjected
300 to conventional single UV-C irradiation showed a tendency to maintain higher firmness than the
301 control the differences were not statistically significant (**Figure 4**). **Cote et al. (2013)** found that
302 for single application of 4 kJ m^{-2} the efficacy of UV-C applications in firmness retention is
303 highly dependent on the radiation intensity. Previous work showed that UV may delay
304 strawberry softening. However, the effect was much more limited than that reported for other
305 physical treatments such as hot air conditioning or high CO_2 atmospheres. Both low dose

306 fractionated UV treatments improved firmness retention. The delay in fruit softening of cyclic
307 low dose UV-C treatments was still observed after 13 days of storage (**Figure 4**). The biological
308 basis of the imporved texture of low fluence *two-* and *multi-step* treatments occurs deserves
309 further studies. Down-regulation of genes coding for cell wall degrading proteins by pre-storage
310 UV irradiation has been reported (**Pombo et al., 2009)**. In this case the inhibitory effect was
311 transient, and normal mRNA levels and enzyme activities recovered after few days. In this
312 scenario if low UV-C doses used for cyclic irradiation are sufficient to disturb normal ripening
313 expression pattern is plausible to hypothesize that the inhibitory effect the biochemical
314 determinants of fruit softening be sustained longer. The effect of UV-C treatment schedule on
315 fruit cell wall degrading proteins has not been reported and deserves further analysis. In any
316 case, it would be valuable to establish the minimal inhibitory treatment conditions (dose and
317 intensity) and the interval between photochemical treatments. In any case, the improved efficacy
318 of fractionated treatments to maintain firmness has great interest given that excessive softening
319 is one of the main factors limiting the postharvest life of strawberry fruit.

320 We also evaluated the residue obtained the residue after extensive extraction in boiling
321 ethanol (AIR) which for fruits having low starch levels represents mainly the insoluble cell wall
322 material. Before cold storage the AIR ranged between 1.87 and 2.01% without differences
323 among treatments. No significant changes were found in the AIR of control fruit. In contrast
324 strawberries subjected to fractionated UV exposure showed an increasing trend (**Table 3**). The
325 increase of insoluble material is at least unexpected given that it is know that extensive
326 polysaccharide degradation accompanies fruit softening (**El Ghaouth et al. 2003**),. UV-C
327 treatments are known to induce the formation of reactive oxygen species such as H_2O_2 (**Civello**
328 **et al. 2006**) which could contribute to the formation of cross links within the cell walls.
329 Oxidative coupling phenolics, and hydroxyproline and tyrosine in wall proteins in response to
330 fungal attack has been reported (**Bradley et al. 1992; Charles et al., 2008**). The oxidative
331 cross-linking of cell wall structural proteins is thought to be a rapid defense response to
332 strengthen the cell wall against the invading pathogen prior to the activation of other post-
333 transcription dependent defense responses (**Brisson et al. 1994**). The higher levels of AIR in

cyclic low dose UV treated fruit suggests that the improved maintenance of fruit cell wall integrity contributes to reduce fruit susceptibility to pathogen attack as has been shown in other ripening fruits (**Cantu et al. 2008**).

3.5 Sensory visual evaluation

We finally conducted a sensory evaluation panel to evaluate whether untrained consumers would detect any differences among control and UV treated strawberries that may affect their purchase decision. After 10 days of storage fruit subjected to *two-step* UV-C irradiation had the highest scores in fruit color, freshness and overall acceptability (**Figure 5**). Despite of the lack of differences in instrumental color values consumers preferred UV treated fruit. Based on further analysis of such discrepancy the highest panelists score for irradiated fruits was due to higher gloss which may be more directly related to surface dehydration than to pigment contents. Scores for all the attributes after 13 d of storage were dramatically higher for two-*step* and *multi-step* treatments given the reduced decay and dehydration observed in these groups (data not shown).

CONCLUSIONS

UV-C treated strawberries showed, after 13 d at 0 °C, lower respiration than the control, suggesting that fruit deterioration was reduced. UV-C exposure also caused a marked decrease in decay, molds, weight loss and softening; with the effect being significantly greater in fruit subjected to *two step* and *multi-step* treatments. Instead, the UV-C irradiation schedule did not affect acidity, sugars and ascorbic acid content. Repeated low dose UV exposure was more effective to yeast counts than single pre-storage high fluence irradiation. M*ulti-step* treated strawberries showed an increase in alcohol insoluble material during storage indicating that repeated UV-C irradiation may be inducing *de novo* deposition and/or cross linking of cell wall material. Finally subjected to *two-step UV* showed highest sensory scores in calyx color,

freshness and acceptability when presented to non-trained consumers. Overall, results show that cyclic low dose UV-C treatments retain strawberry fruit quality more effectively than conventional pre-storage single high fluence applications.

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FIGURE CAPTIONS

Figure 1. Weight loss in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of *$P<0.05$. *Control:* Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

Figure 2. Decay in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of *$P<0.05$. *Control:* Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

Figure 3. Viable colony counts of mold and yeast in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of *$P<0.05$. *Control:* Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

542 **Figure 4.** Firmness in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different
543 letters indicate differences based on a Tukey test at a level of significance of *$P<0.05$. *Control:*
544 Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage
545 (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five
546 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

547

548 **Figure 5.** Sensory scores for color, freshness appearance and overall acceptability in strawberry
549 fruit stored at 0 °C for 10 days. Different letters indicate differences based on a Tukey test at a
550 level of significance of *$P<0.05$. *Control:* Without UV-C application (■); *One-step UV:* single
551 UV-C 4 kJ m^{-2} application before storage (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0
552 and 4 d of storage (■) and *Multi-step*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

553

554

Table 1: Receptacle and calyx color in control or irradiated strawberry fruit **(***one-step UV*: single UV-C 4 kJ m^{-2} application before storage; *two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage and *multi-step UV*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage) during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of *$P<0.05$.

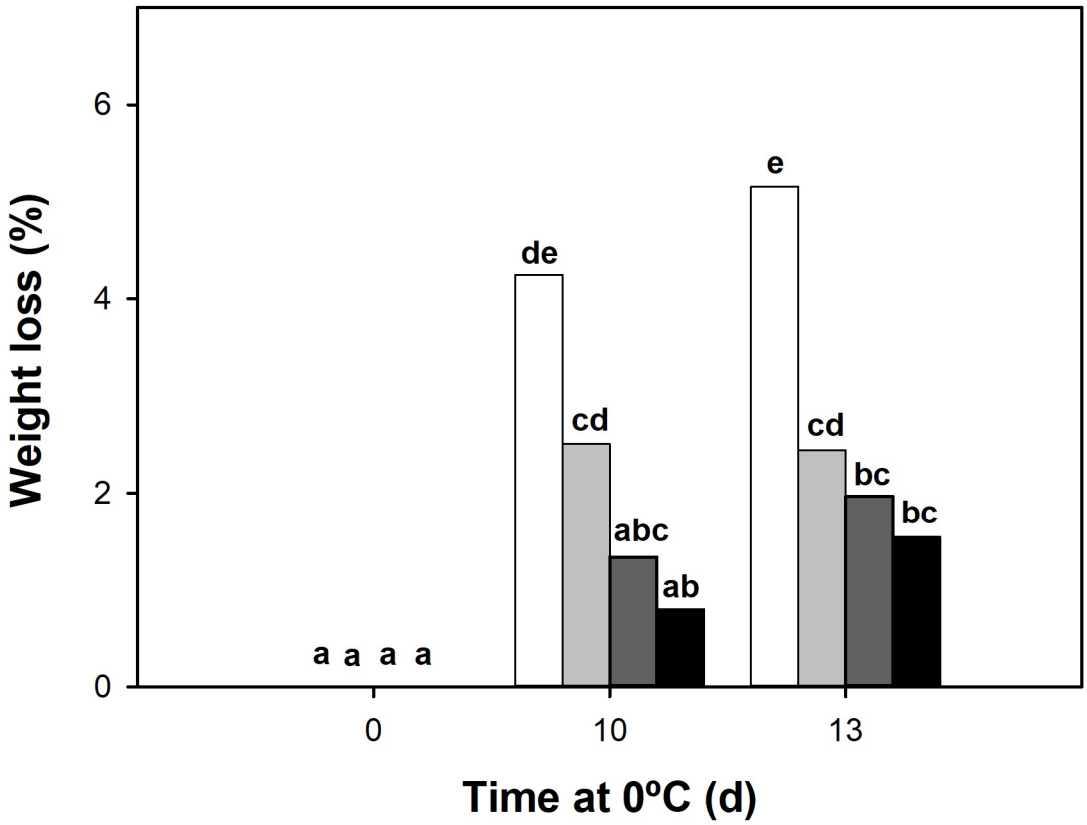
		Time at 0 °C (d)					
		0		10		13	
Receptacle lightness (L*)	Control	51.9g	*±3.2*	47.7e	*±2.0*	44.9a	*±3.9*
	One-step UV	49.9f	*±2.9*	46.9cde	*±2.5*	45.3ab	*±4.4*
	Two-step UV	50.6f	*±2.8*	46.9de	*±2.6*	46.3bcd	*±2.2*
	Multi-step UV	50.5f	*±2.9*	46.8cde	*±1.9*	45.0abc	*±3.3*
Receptacle ºHue	Control	47.5ef	*±5.1*	28.4ab	*±3.8*	31.8c	*±4.0*
	One-step UV	45.8d	*±4.3*	29.8ab	*±3.9*	28.6ab	*±4.9*
	Two-step UV	46.2de	*±3.9*	28.3ab	*±8.5*	29.9bc	*±5.2*
	Multi-step UV	47.9f	*±4.6*	28a	*±4.0*	29.8ab	*±4.6*
Calyx lightness (L*)	Control	51.1d	*±3.1*	49.4c	*±2.6*	47.2a	*±4.0*
	One-step UV	49.4c	*±2.5*	48.7abc	*±3.4*	48.7abc	*±3.4*
	Two-step UV	50.1c	*±2.8*	47.9ab	*±2.8*	49.3bc	*±2.9*
	Multi-step UV	50c	*±2.9*	49.4c	*±1.7*	49.1bc	*±2.8*
Calyx ºHue	Control	134d	*±4.3*	119.3ab	*±7.9*	121.7b	*±6.6*
	One-step UV	133.5cd	*±4.3*	116.4a	*±9.2*	116.6a	*±12.9*
	Two-step UV	134.2d	*±3.4*	120.7ab	*±10.5*	119.4ab	*±6.1*
	Multi-step UV	132.1c	*±5.1*	120.8b	*±7.2*	118.0ab	*±10.8*

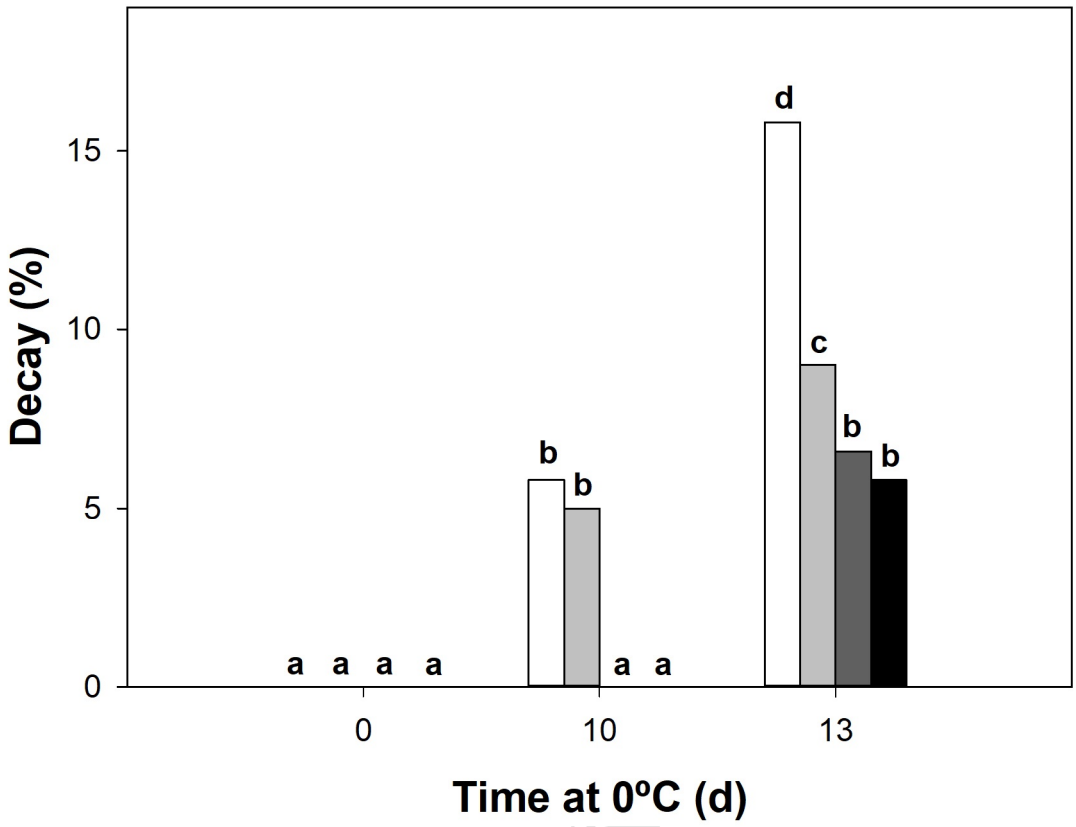
Table 2: Respiration rate, acidity, glucose, fructose, sucrose and ascorbic acid content in control or irradiated strawberry fruit (*one-step UV*: single UV-C 4 kJ m^{-2} application before storage; *two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage and *multi-step UV*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage) during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of $*P<0.05$.

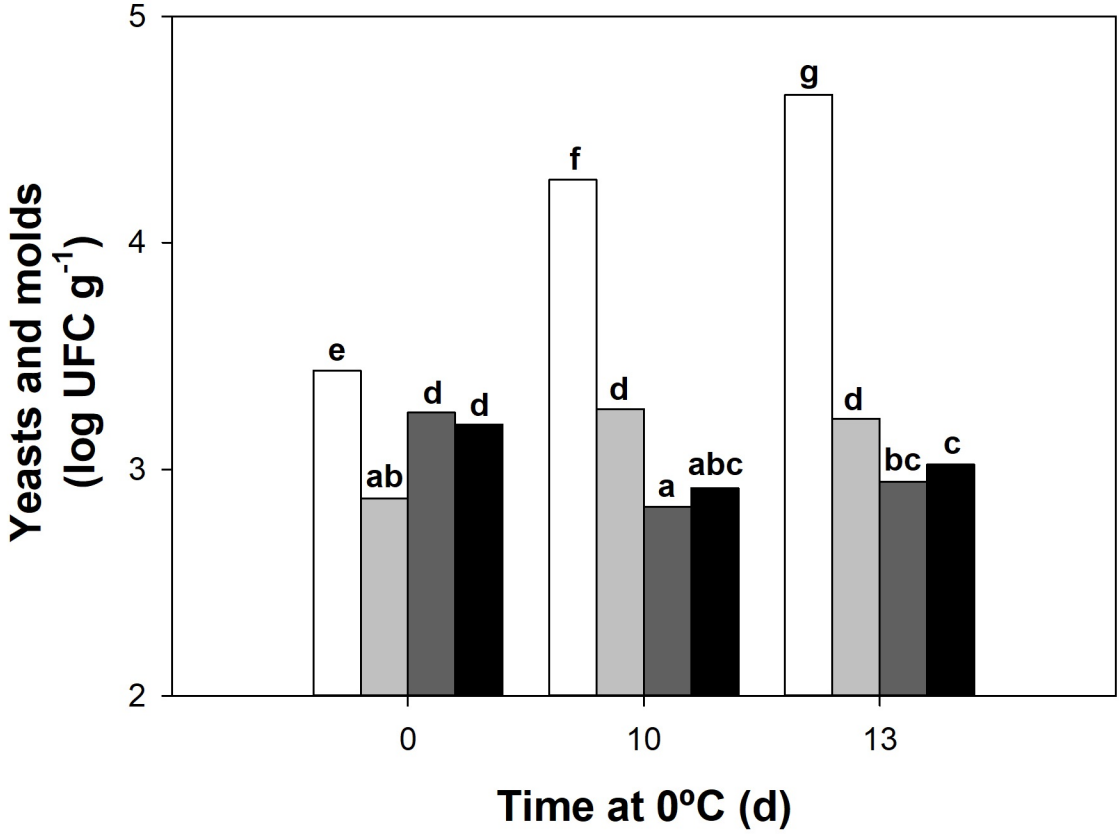
		Time at 0 °C (d)					
		0		**10**		**13**	
Respiration rate (mL kg^{-1} h^{-1})	Control	24.4ab	*± 8.3*	45.5b	*±9.1*	61.1c	*±1.2*
	One-step UV	29.8a	*± 0.5*	49.8b	*±10.3*	50.3b	*±9.0*
	Two-step UV	30.3a	*±0.4*	53.6b	*±3.4*	54.7b	*±4.7*
	Multi-step UV	23.2a	*±11.0*	52.2b	*±0.6*	51.1b	*±7.7*
Acidity (meq. H^+ kg)	Control	0.3ab	$\pm 0.3x10^{-2}$	0.3ab	$\pm 0.9x10^{-2}$	0.3a	$\pm 0.3x10^{-2}$
	One-step UV	0.4 c	$\pm 1.8x10^{-2}$	0.3ab	$\pm 2.3x10^{-2}$	0.3ab	$\pm 3.7x10^{-2}$
	Two-step UV	0.3bc	$\pm 3.7x10^{-2}$	0.3ab	$\pm 1.3x10^{-2}$	0.3ab	$\pm 0.4x10^{-2}$
	Multi-step UV	0.3ab	$\pm 0.2x10^{-1}$	0.3ab	$\pm 1.3x10^{-2}$	0.3ab	$\pm 0.1x10^{-3}$
Glucose (%)	Control	1.2abc	*±0.3*	1.5c	*±0.1*	1.5c	$\pm 4.3x10^{-2}$
	One-step UV	1.1ab	*±0.1*	1.3bc	*±0.1*	1.4bc	*±0.1*
	Two-step UV	1.0a	*±0.2*	1.2abc	*±0.1*	1.5c	*±0.2*
	Multi-step UV	1.0a	*±0.1*	1.2abc	*±0.4*	1.36c	*±0.1*
Fructose (%)	Control	1.2ab	*±0.1*	1.5c	$\pm 3.7x10^{-2}$	1.6c	*±0.1*
	One-step UV	1.1ab	*±0.1*	1.4bc	*±0.1*	1.5c	$\pm 8.2x10^{-2}$
	Two-step UV	1.1a	*±0.1*	1.3abc	*±0.2*	1.6c	*±0.1*
	Multi-step UV	1.0a	*±0.3*	1.3abc	*±0.4*	1.5c	*±0.1*
Sucrose (%)	Control	1.5c	*±0.3*	1.1a	*±0.166*	0.7a	*±0.2*
	Single-step UV	1.5c	$\pm 9.7x10^{-2}$	1.0ab	*±0.2*	0.8ab	*±0.1*
	Two-step UV	1.5c	*±0.2*	0.9ab	*±0.2*	0.8ab	$\pm 7x10^{-3}$
	Multi-step UV	1.5c	*±0.1*	0.8ab	*±0.1*	0.8ab	*±0.1*
Ascorbic acid (mg 100 g^{-1})	Control	34.7a	*±1.2*	46.1abc	*±3.3*	35.0a	*±4.6*
	Single-step UV	41.6abc	*±1.2*	52.2c	*±5.1*	38.0ab	*±9.9*
	Two-step UV	39.8bc	*±1.7*	40.0ab	*±10.8*	46.1abc	*±8.2*
	Multi-step UV	45.2abc	*±1.3*	48.1bc	*±1.5*	44.8abc	*±2.6*

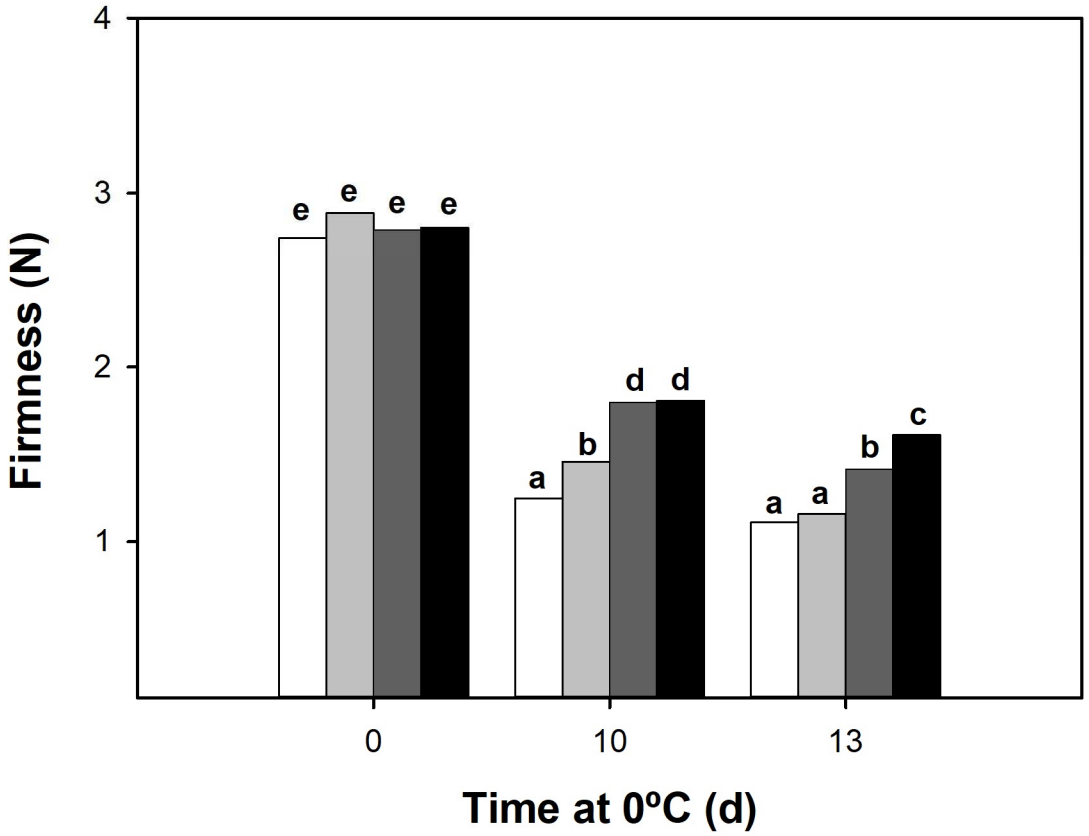
Table 3: AIR **(***one-step UV*: single UV-C 4 kJ m^{-2} application before storage; *two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage and *multi-step UV*: five 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage) during storage at 0 °C for 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of significance of **P<0.05*.

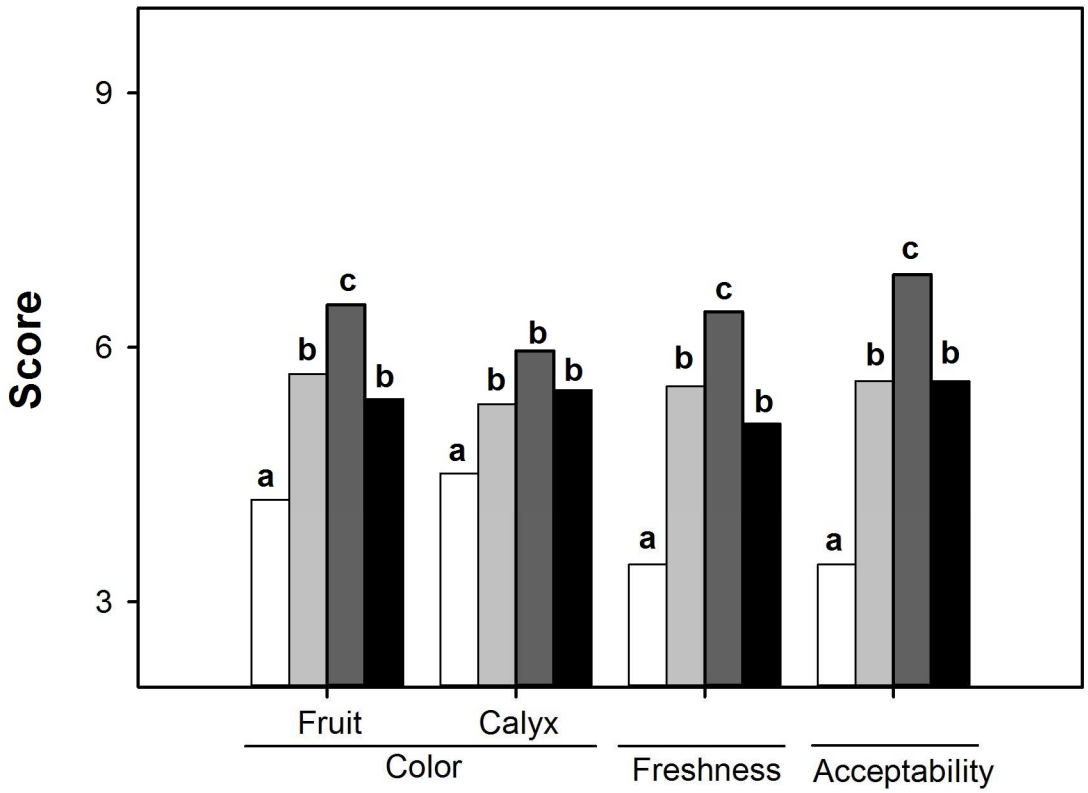
		Time at 0 °C (d)					
		0		**10**		**13**	
AIR (g 100g^{-1})	Control	1.81ab	*±0.22*	1.87ab	*±0.04*	2.05bc	*±0.17*
	One-step UV	2.00ab	*±0.0*	1.83ab	*±0.03*	2.27cd	*±0.08*
	Two-step UV	1.99ab	*±0.21*	1.77a	*±0.08*	2.42d	*±0.03*
	Multi-step UV	1.85ab	*±0.04*	2.01abc	*±0.15*	2.44d	*±0.08*











Highlights

- Low-dose cyclic UV_C exposure extended the postharvest life of refrigerated strawberry
- Two and multi-step UV-C irradiation maintained firmness and markedly reduced decay
- Fruit exposed to repeated low dose irradiation showed highest consumer sensory scores
- For a fixed total dose the irradiation schedule has great impact on the efficacy of UV-C treatments
- Repeated low dose exposure was more effective than conventional single-step irradiation