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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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2 **effectively than conventional pre-storage single high fluence applications**

3

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22 **Abstract**

23 UV-C irradiation has been shown to reduce fruit decay and delay ripening. Based on an

24 expected higher impact and applicability, UV irradiation treatments have been almost

25 exclusively done before storage at relatively high doses. We evaluated the influence of the

26 pattern of repeated short dose UV-C exposure on quality maintenance of strawberry fruit.

27 Strawberries were subjected to the following treatments: S*ingle-step UV*: single 4 kJ m^{-2}

28 irradiation prior to storage; *two-step UV*: two consecutive 2 kJ m^{-2} UV irradiations at harvest

29 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

30 storage respectively. A non-irradiated group was left untreated. Samples were stored at 0 °C for

31 13 days. All UV-C treatments decreased decay, weight loss and softening. The quality retention

32 was higher in fruit subjected *two-step* and *multi-step* UV-C. Multiple low dose UV exposure

33 reduced calyx browning more efficiently. Repeated low UV-C dose decreased mold and yeast

34 counts to a higher extent. *Multi-step* UV treated fruit showed higher alcohol insoluble residue.

35 *Two-step* UV-C treated fruit showed the highest sensorial scores. Repeated low dose UV-C

36 treatments are more effective in preventing strawberry fruit than conventional single high-

37 fluence pre-storage irradiation.

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

39

40 **1. INTRODUCTION**

41   Strawberries are widely appreciated for their bright red color, unique aroma and texture

42 and high antioxidant capacity (**Giné-Bordonaba and Terry 2016; Li et al. 2017**). However,

43 continuous distribution of premium berries is challenging due to their high perishability (**Bower**

44 **et al. 2003; Chen et al. 2016**). Though low temperature storage effectively reduce deterioration,

45 even under proper temperature management strawberry shelf-life life rarely exceeds 7-10 days

46 (**Erkan et al. 2008**). To date, no postharvest fungicides have been approved to control *Botrytis*

47   *cinerea* and other wet treatments such as washing are not recommended since they may increase

48   decay susceptibility (**Mitcham 2016**). Firming agents such as calcium salts (**Aguayo et al.**

49   **2008**) which has been effectively shown to delay softening and fungal attack is mainly limited

50   to the fresh-cut industry whereas strategies such as surface coating showing good results at lab

51   scale (**Romanazzi et al. 2016**) have had difficulties to be up-scaled to commercial settings.

52   Consequently, there is a relatively limited set of strategies to reduce postharvest losses in

53   strawberry limited.

54   In recent years there have been great interests in the search for non-chemical dry

55   methods that can prevent fruit deterioration (**Vicente and Lurie 2014**). Several research groups

56   have started to evaluate UV-C treatments as a potential alternative to control spoilage (**Civello**

57   **et al. 2006; Xu et al. 2017**). Strawberry pre-storage UV-C treatments at doses ranging from 0.2

58   to 4.2 kJ m^{-2} reduced decay (**Baka et al. 1999; Erkan et al. 2008; Li et al. 2014; Xu et al.**

59   **2017**). UV-C radiation has been shown to affect fungal metabolism (**Bintsis et al. 2000;**

60   **Trivittayasil et al. 2016**). **Pan et al. (2004)** reported that UV-C radiation (4 kJ m^{-2}), reduced the

61   rate of germination of *Botrytis* and *Rhizopus* conidia. Besides its direct effect on plant

62   pathogens, UV-C radiation has been shown to modulate ripening-associated processes such as

63   softening (**Stevens et al. 2004; Pan et al. 2004**) and to elicit the accumulation of antioxidants

64   (**Erkan et al. 2008**) and phytoalexins (**D´hallewin et al. 1999**). **Pombo et al. (2011)** reported

65   that UV-C irradiation may increase the expression of chitinases and β-1,3-glucanases. Early

66   work by **Nigro et al. (2000)** also reported the induction phenylpropanoid regulatory enzymes

67   such as phenylalanine ammonia lyase (PAL).

68   Several factors determining the efficacy of UV treatments have been studied; the

69   maturity stage at which the fruit is irradiated influenced the outcome of UV treatments, with

70   early applications having more dramatic effect delaying ripening (**Liu et al. 1993; Charles et**

71   **al. 2002**). However, strawberries must be picked at complete maturity in order to attain full

72   flavor, what narrows the window at which UV treatments could be applied. The UV radiation

73   dose (fluence) applied also affects the benefits of UV-C treatments on fruit quality maintenance.

74   This has been, by far the variable most extensively studied (**Civello et al. 2014**). **Cote et al.**

75 **(2013)** showed that for a given dose radiation intensity also affects the efficacy of UV-C

76 treatments. Other factors such as the as the pattern of UV exposure have been barely studied.

77 Even though pre-storage applications would be more practical than cyclic UV-C understanding

78 the responses of fruit to different irradiation conditions is very important to better understand

79 the physiological effects of postharvest photochemical treatments. No studies have been

80 conducted to determine if small point applications throughout the storage period could improve

81 quality retention relative to conventional single high fluence UV treatments. The aim of this

82 work was to determine if repeated short applications throughout the storage period could

83 improve quality retention relative to conventional single high fluence UV treatments

84

85 **2 MATERIALS & METHODS**

86

87 **2.1 Plant material, treatments and storage conditions**

88   Strawberry fruit (*Fragaria* x *ananassa* cv Camarosa) grown in La Plata, Argentina was

89 harvested at commercial maturity and immediately transported to the laboratory. Fruit was put

90 in polyethylene terephthalate (PET) trays, in groups of 10 and was located under an irradiation

91 mobile bank (1.7 m x 0.8 m) consisting a closed cabinet containing on the upper side 12 UV-C

92 lamps (UV-C peak emission at 254 nm, TUV G30T8, 30 W, Philips, Argentina) with a global

93 maximum radiation intensity of 38 W m^{-2}. The fruit was rotated in order to irradiate two

94 opposite sides. Fruit was irradiated at a distance of 30 cm. UV-C radiation dose was evaluated

95 by using a digital UV-C radiometer (ElectroLite Miodel LC 300, USA) located in the central

96 zone of the irradiation zone. The following treatments were applied:

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

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

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

100 One set of non-irradiated fruit was used as a *Control*. Samples were covered with a perforated

101 plastic lid and stored 0, 10 or 13 days at 0 ºC. For those treatments requiring UV exposure

102 during the storage period the bank was used directly into the storage area to avoid oscillations in

103 fruit temperature. Samples were immediately analyzed after sampling or otherwise frozen in

104 liquid N_2 and stored at -80 °C until analysis. Four trays containing 10 fruit each were used for

105 every treatments and storage time. The whole experiment was repeated three times.

106

107 **2.2. Respiration rate**

108 Samples were taken and held at 20 °C until thermal equilibration. Ten fruits were placed

109 in a 1.5 L glass jar which was hermetically sealed. An IR sensor (Alnor, USA) was used to

110 determine the change in CO_2 in the headspace during a 20 min period. The respiration rate was

111 calculated by determining the mass of CO_2 produced per kg of fruit in an hour. Three

112 measurements were done for each treatment and sampling date.

113

114 **2.3. Weight loss**

115 Individual fruits were weighed at the beginning of the storage period and after 10 or 13

116 d at 0 °C. Weight loss was calculated as: $WL = 100 \times (W_i - W_f)/Wi$, being W_i the initial sample

117 weight and W_f the final weight. Results were expressed in percentage.

118

119 **2.4. Decay**

120 The percentage of fruit showing incipient symptoms of decay (local tissue maceration)

121 or excessive softening on each tray was recorded. Decay incidence was expressed as percentage

122 of decayed fruits. Four trays containing 10 fruit each were evaluated for each treatment and

123 storage time.

124

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

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

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 °C; 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

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 °C. 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[TM] 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

2.13. Statistical analysis

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

206

3. RESULTS AND DISCUSSION

3.1 Weight loss, decay, microbial counts and phenolic compounds

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

224   After 10 days at 0 °C no differences in fruit decay were found between control and

225   conventionally (*one-step*) UV-C treated strawberries. In contrast, the both *two-step* and *multi-*

226   *step UV-C* treated fruit showed no decay (**Figures 2**). After 13 days at 0 °C a rapid increase in

227   fruit decay was found in control strawberries. At this sampling date, fruit subjected to single

228   pre-storage UV irradiation presented lower decay incidence than the corresponding control. This

229   is coincident with previous work showing that single UV irradiation at doses ca. 4 kJ m^{-2} can be

230   useful to control decay by *Botrytis cinerea* (**Pan et al. 2004; Cote et al. 2013**). Interestingly,

231   also after long term storage *two-* and *multi-step* applications relying on repeated exposure at low

232   radiation fluence were significantly more effective than single pre-storage irradiation to control

233   decay.

234   We subsequently evaluated the viable count of molds and yeast (**Figure 3**). The colonies

235   counted represented mostly yeasts. At harvest 3.4 CFU g^{-1} were found. Immediately after the

236   initial irradiation there was a significant decrease in yeast counts all the treatments. The greatest

237   reduction was observed in one step treatments receiving the highest radiation dose at day 0 (4 kJ

238   m^{-2}). Work by Mercier et al. has shown that direct inactivation of microorganisms by UV

239   radiation is highly dependent on radiation dosage (**Mercier et al., 2001**). The fractionated *two-*

240   *step* and *multi-step* treatments showed no differences in yeast counts reduction prior to storage.

241   In this case, a significant but modest reduction (*ca*. 0.25 log cycles) was observed. The counts of

242   control strawberries increased one log cycle during 10 days of storage. Fruit subjected to *one-*

243   *step* UV irradiated also showed an increasing trend. Remarkably, the counts in fruit subjected to

244   low fluence *two-* and *multi-step* UV-C showed no changes in yeast counts throughout the

245   storage period, but rather a decrease. This shows that, for a similar total radiation dosage,

246   repeated low dose UV-C exposure *in vivo* resulted in a better more effective reduction in yeasts

247   CFU than one step irradiation. One plausible explanation is that the repeated irradiation, even

248   with low partial doses, was sufficient to exert inhibitory effects on yeast viability and that

249   several treatments was more detrimental (**Nhung et al. 2012; Sinha and Häder 2002**). Despite

250   of the potential direct effect that repeat low dose UV exposure could exert on fruit pathogens we

251   cannot exclude that fractionated UV could have induced defensive responses. Early work by

252   **Ben-Yehoshua (1992)** clearly showed that UV-C irradiation induced the accumulation of the

253   phytoalexin scoparone in citrus flavedo. Subsequent studies in even in strawberry reported that

254   UV-C irradiation also increased the activity of enzymes related to active responses such as

255   chitinases and β-1,3 glucanases (**Pombo et al. 2011**) or associated with the biosynthesis of

256   antimicrobial phenolics (**Nigro et al., 2000; Erkan et al. 2008**). For successful colonization, a

257   pathogen must succeed over the fruit's defensive arsenal. This could be done even for a single

258   microorganism by many different mechanisms depending on the prevailing physiological and

259   environmental conditions. **Prusky et al., (2016)** recently suggested that carbon availability in

260   the environment is a key factor triggering the production and secretion of ammonia and organic

261   acids which could modulate the pH and result in completely different pathogenic responses.

262   Then, it would be interesting to evaluate whether the pattern of exposure to UV-C radiation

263   could affect carbon status within the apoplast and contribute to affect pathogen invasion.

264

265   **3.2 Receptacle and calyx color, respiration, sugars, acidity and ascorbic acid**

266   Fruit receptacle hue and lightness decreased indicating that ripening progressed even

267   during storage at 0 °C (**Table 1**). The receptacle hue decreases during storage from 45º at the

268   beginning to 30º at the end of storage period. No differences in hue values were recorded

269   between control and UV irradiation fruit for any treatment schedule evaluated (**Table 1**). This is

270   consistent with the results reported by **Pan et al. (2004)** who found subtle color changes in UV-

271   C treated strawberries. The UV treatments induced a slight reduction in receptacle lightness

272   (L*). However, this effect was very limited compared to the browning recorded during the 13-d

273   storage period. At the last sampling date, the *two-step UV-C* treatments caused lower lightness

274   loss than control (**Table 1**). Calyx hue angle dropped in all treatments from values *ca.* 130 at

275   harvest to 120 at the last sampling date in association with chlorophyll degradation. In

276   accordance with **Marquenie et al. (2002)** we did find some calyx browning and drying in UV-C

277   treated strawberries. The reduction of calyx L* values was delayed in *two-* and *multi-step* UV

278   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 w

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

334   cyclic low dose UV treated fruit suggests that the improved maintenance of fruit cell wall

335   integrity contributes to reduce fruit susceptibility to pathogen attack as has been shown in other

336   ripening fruits (**Cantu et al. 2008**).

337

338   **3.5 Sensory visual evaluation**

339   We finally conducted a sensory evaluation panel to evaluate whether untrained

340   consumers would detect any differences among control and UV treated strawberries that may

341   affect their purchase decision. After 10 days of storage fruit subjected to *two-step* UV-C

342   irradiation had the highest scores in fruit color, freshness and overall acceptability (**Figure 5**).

343   Despite of the lack of differences in instrumental color values consumers preferred UV treated

344   fruit. Based on further analysis of such discrepancy the highest panelists score for irradiated

345   fruits was due to higher gloss which may be more directly related to surface dehydration than to

346   pigment contents. Scores for all the attributes after 13 d of storage were dramatically higher for

347   two-*step* and *multi-step* treatments given the reduced decay and dehydration observed in these

348   groups (data not shown).

349

350   **CONCLUSIONS**

351   UV-C treated strawberries showed, after 13 d at 0 °C, lower respiration than the control,

352   suggesting that fruit deterioration was reduced. UV-C exposure also caused a marked decrease

353   in decay, molds, weight loss and softening; with the effect being significantly greater in fruit

354   subjected to *two step* and *multi-step* treatments. Instead, the UV-C irradiation schedule did not

355   affect acidity, sugars and ascorbic acid content. Repeated low dose UV exposure was more

356   effective to yeast counts than single pre-storage high fluence irradiation. M*ulti-step* treated

357   strawberries showed an increase in alcohol insoluble material during storage indicating that

358   repeated UV-C irradiation may be inducing *de novo* deposition and/or cross linking of cell wall

359   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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521

FIGURE CAPTIONS

523 **Figure 1.** Weight loss in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different

524 letters indicate differences based on a Tukey test at a level of significance of *P<0.05*. *Control:*

525 Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage

526 (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five

527 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

528

529 **Figure 2.** Decay in strawberry fruit during storage at 0 °C for 0, 10 and 13 days. Different

530 letters indicate differences based on a Tukey test at a level of significance of *P<0.05*. *Control:*

531 Without UV-C application (■); *One-step UV:* single UV-C 4 kJ m^{-2} application before storage

532 (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of storage (■) and *Multi-step*: five

533 0.8 kJ m^{-2} after 0, 2, 4, 6 and 8 d of storage (■).

534

535 **Figure 3.** Viable colony counts of mold and yeast in strawberry fruit during storage at 0 °C for

536 0, 10 and 13 days. Different letters indicate differences based on a Tukey test at a level of

537 significance of *P<0.05*. *Control:* Without UV-C application (■); *One-step UV:* single UV-C 4

538 kJ m^{-2} application before storage (■); *Two-step UV*: two 2 kJ m^{-2} applications after 0 and 4 d of

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

540

541

542 **<u>Figure 4.</u>** 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 **<u>Figure 5.</u>** 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	±0.3x10^{-2}	0.3ab	±0.9x10^{-2}	0.3a	±0.3x10^{-2}
	One-step UV	0.4 c	±1.8x10^{-2}	0.3ab	±2.3x10^{-2}	0.3ab	±3.7x10^{-2}
	Two-step UV	0.3bc	±3.7x10^{-2}	0.3ab	±1.3x10^{-2}	0.3ab	±0.4x10^{-2}
	Multi-step UV	0.3ab	±0.2x10^{-1}	0.3ab	±1.3x10^{-2}	0.3ab	±0.1x10^{-3}
Glucose (%)	Control	1.2abc	±0.3	1.5c	±0.1	1.5c	±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	±3.7x10^{-2}	1.6c	±0.1
	One-step UV	1.1ab	±0.1	1.4bc	±0.1	1.5c	±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	±9.7x10^{-2}	1.0ab	±0.2	0.8ab	±0.1
	Two-step UV	1.5c	±0.2	0.9ab	±0.2	0.8ab	±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*.
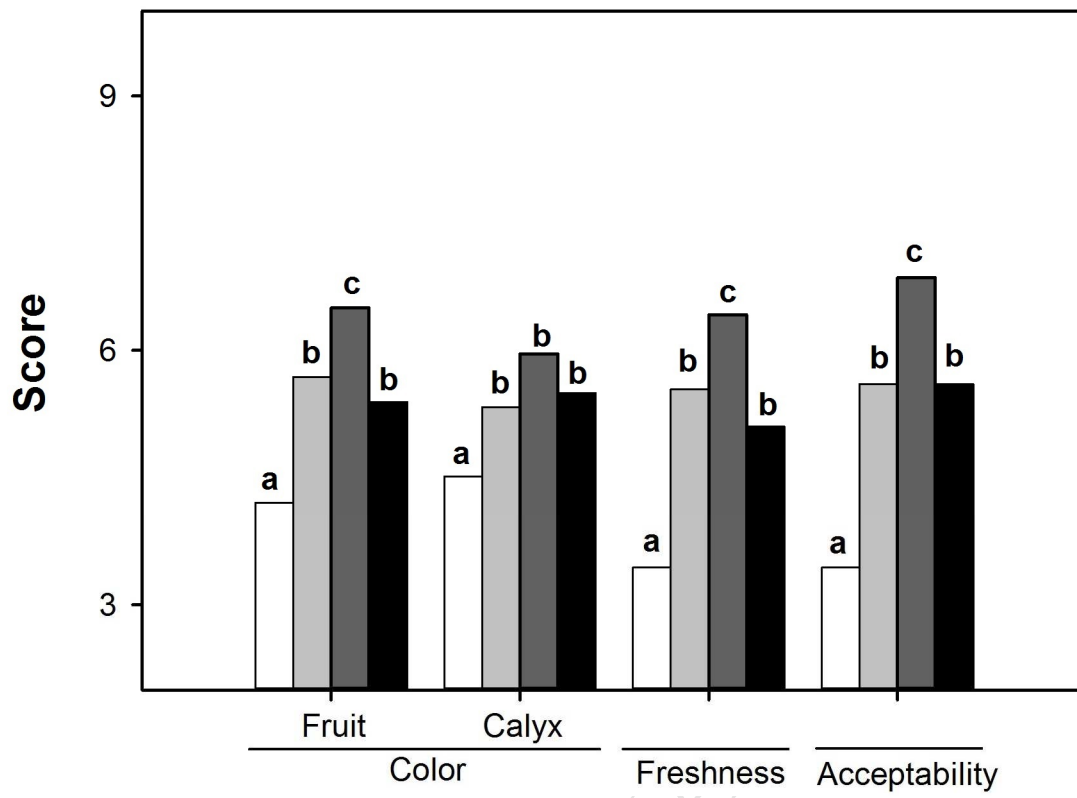
		Time at 0 °C (d)					
		0		**10**		**13**	
AIR (g 100g^{-1})	Control	1.81ab	*±0.22*	1.87ab	*±0.04*	2.05bc	*±0.17*
	One-step UV	2.00ab	*±0.0*	1.83ab	*±0.03*	2.27cd	*±0.08*
	Two-step UV	1.99ab	*±0.21*	1.77a	*±0.08*	2.42d	*±0.03*
	Multi-step UV	1.85ab	*±0.04*	2.01abc	*±0.15*	2.44d	*±0.08*
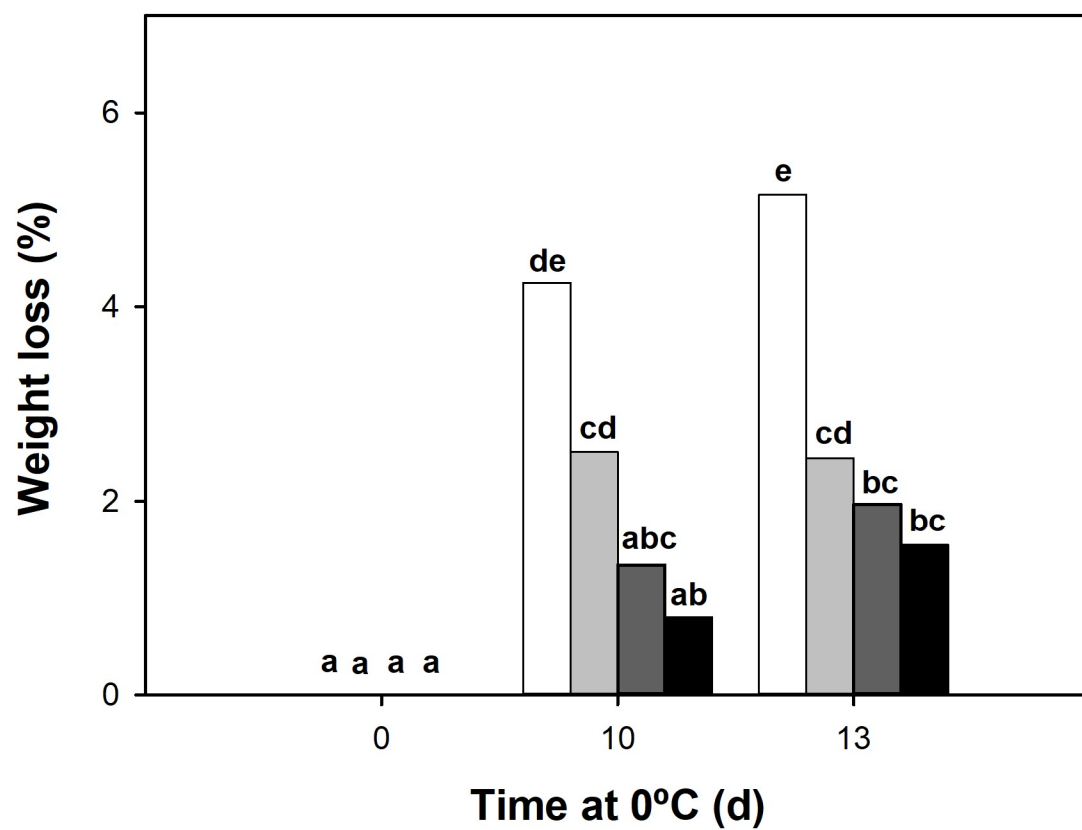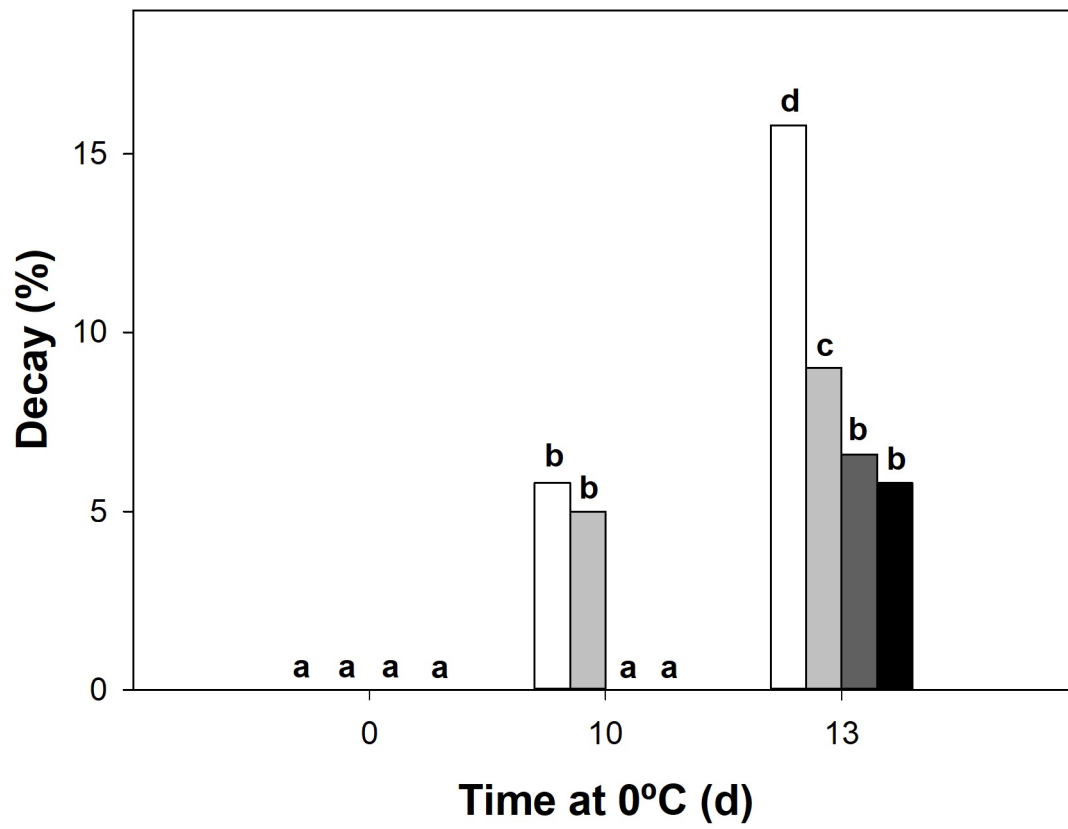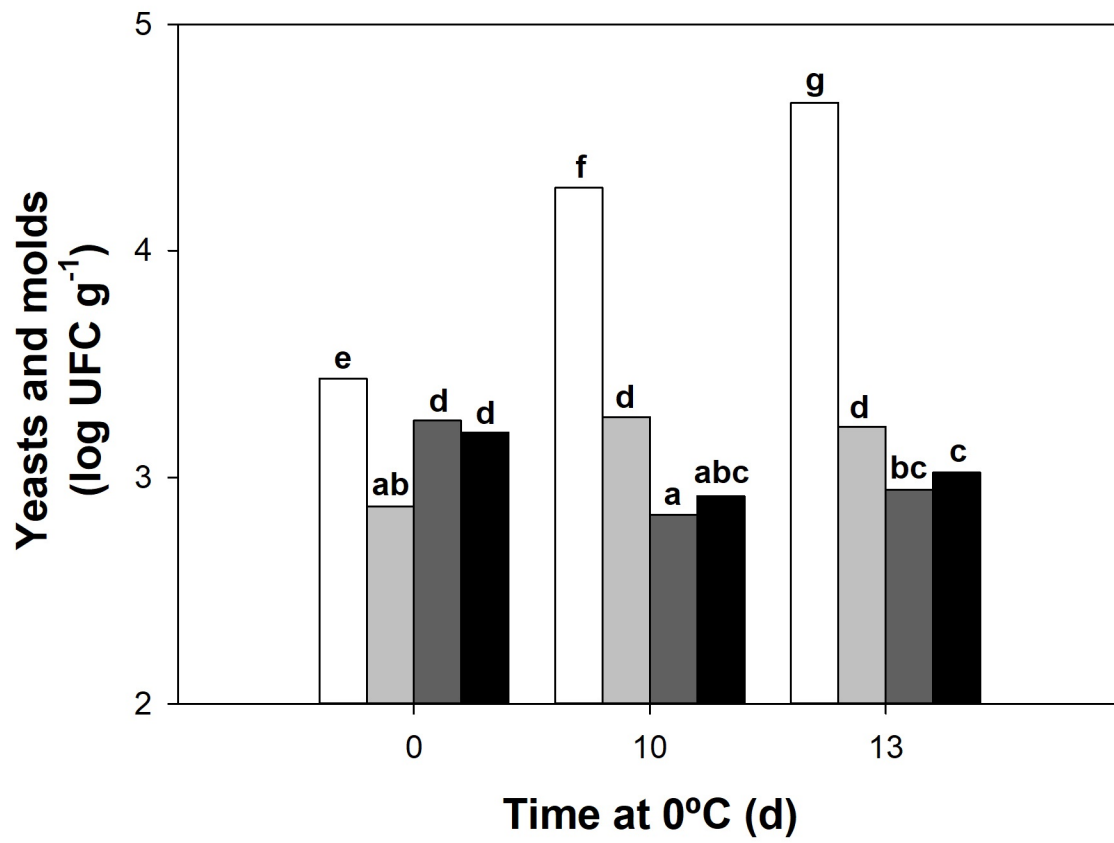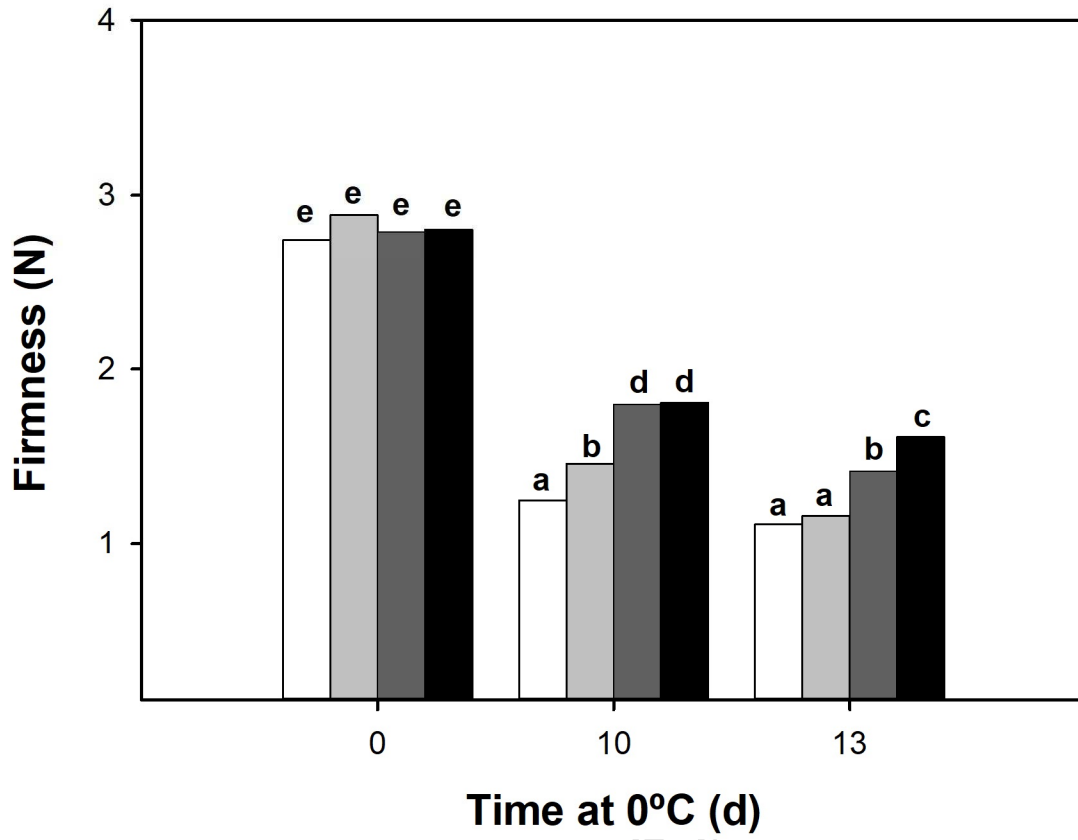
Highlights

- 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