

Possibilities of modified atmosphere packaging to prevent the occurrence of internal fruit rot in bell pepper fruit (*Capsicum annuum*) caused by *Fusarium* spp

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1 **POSSIBILITIES OF MODIFIED ATMOSPHERE PACKAGING TO PREVENT THE OCCURRENCE OF**
2 **INTERNAL FRUIT ROT IN BELL PEPPER FRUIT (*CAPSICUM ANNUUM*) CAUSED BY *FUSARIUM* SPP.**

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17 **ABSTRACT: Bell pepper (*Capsicum annuum* L.), with its wide array of colors and**
18 **flavors, plays an important role in many different cuisines around the world. Yet**
19 **once harvested, it is a highly perishable fruit and needs appropriate post-harvest**
20 **handling. Recently, post-harvest internal rotting (IFR) by *Fusarium lactis* species**
21 **complex isolates (FLASC), became an additional challenge to maintain shelf-life**
22 **and quality of bell pepper fruit. Therefore, modified atmosphere packaging (MAP)**
23 **was explored as a possible technique to postpone symptom development of**
24 **infected bell peppers. Four artificially infected bell pepper cultivars with different**

25 **susceptibility towards IFR were stored under MAP conditions for a maximum of**
26 **14 d at challenging conditions of 20 °C resembling unrefrigerated shelf life**
27 **conditions.** Each week, 5 fruit of each object were analyzed for IFR symptom
28 **development and additional physicochemical and quality parameters. For all**
29 **cultivars, MAP packaged fruit showed less severe fungal proliferation compared**
30 **to controls after 14 d. As total titratable acid (TA), total soluble solids (TSS) and**
31 **vitamin C concentrations in fruit remained rather stable throughout the**
32 **experiment, fungal development was likely to be postponed directly due to**
33 **reduced oxygen levels in the pouches rather than a decreased host susceptibility**
34 **by influencing fruit metabolism. Since no significant differences of disease**
35 **development were observed between sensitive and less sensitive cultivars for**
36 **both colors, sensitivity for IFR seems not likely to be caused by different post-**
37 **harvest disease development patterns but rather by differences in the initial**
38 **susceptibility for flower infection under normal growth conditions. Based on our**
39 **results, MAP can indeed be considered a useful tool to ameliorate IFR**
40 **development during post-harvest storage of bell pepper under conventional**
41 **temperatures of 7-16 °C.**

42

43 **1 INTRODUCTION:**

44 The increase in global population and related global demand for vital food needs are
45 challenging goals for food production in the upcoming decennia (Godfray et al., 2010; Porat
46 et al., 2018). Vital foods such as vegetables play an important role in the human diet as
47 the lack of sufficient vegetables has been correlated with mortality rate of pre-school
48 children and other vulnerable groups (Keatinge et al., 2011). An important vegetable, bell
49 pepper (*Capsicum annuum*), is an important source of vitamins, minerals and dietary fibers
50 in many regions of the world (Howard et al., 2000; Bosland et al., 2012; Rubatzky &
51 Yamaguchi, 2012). They are especially known as an excellent source of vitamin C
52 containing the accepted recommended daily allowance of 0.1 – 0.12 g for respectively

53 female and male adults (Bosland et al., 2012; EFSA, 2013; Lee, 2019). In addition, they
54 are also widely used as culinary ingredients due to their color, flavor and pungency (Howard
55 et al., 1994; Frank et al., 2001; Manolopoulou et al., 2010). Therefore, it is not surprising
56 that production and consumption raised with 30 % from 2008 till 2018 and is still rising
57 ("FAOSTAT," 2020).

58 However, this increased production is in jeopardy due to a fungal disease called
59 internal fruit rot (IFR), which can be caused by different members of the *Fusarium* genus
60 but mainly by isolates of the *Fusarium lactis* species complex (FLASC) (Kocks et al., 2004;
61 Yang et al., 2009; Van Poucke et al., 2012). After initial infection of the flower during plant
62 growth, the fungus stays latent in the green unripe fruit until coloration starts (Yang et al.,
63 2010; Frans et al., 2016a). **Reducing resistance of ripening fruit towards pathogens might
64 ensue from disassembling of cell walls, reduction of preformed or induced antifungal
65 compounds and decreasing host defense mechanisms** (Prusky, 1996; Prusky et al., 2013).
66 The increasing acidity and sugar accumulation in the bell pepper fruit upon coloration,
67 might favor the expression of pathogenicity factors **as it has been observed in other
68 pathogen-host complexes** (Luning et al., 1994; Estrada et al., 2000; Castro et al., 2008;
69 Prusky et al., 2016; Liu et al., 2018; Piazzolla et al., 2018; Prusky & Wilson, 2018). In
70 **addition, *Fusarium oxysporum* can adjust its ambient pH depending on the amount of
71 available carbon sources in the host tissue** (Bi et al., 2016). **These factors could potentially
72 benefit IFR development, as FLASC has been reported to tolerate rather high acidity levels
73 of pH 3 very well** (Frans et al., 2017). Initially, the fungus develops a white-pinkish
74 mycelium on the seeds, placenta and inner surface of the infected fruit. In early stages,
75 infected fruit do not show any apparent external symptoms such as sunken lesions until
76 they have progressed to later stages of the supply chain (Utkhede & Mathur, 2004; Yang
77 et al., 2010; Frans et al., 2016b). As infection already takes place before harvest inside
78 the fruit, common post-harvest techniques like surface disinfection with hot water,
79 chlorinated water or UV-light do not result in the prevention of internal fruit rot. Non-
80 destructive methods for analyzing internal fruit quality in real-time mode such as VIS/NIR

81 spectroscopy and X-ray radiography have not been found adequate enough to sort out
82 infected bell pepper fruit mainly due to their internal cavity (Sauviller et al., 2015; Frans
83 et al., 2018).

84 After harvest, storage life of pepper fruit is mainly limited by shriveling associated
85 with rapid water loss, susceptibility to chilling injury and pathological decay (Maalekuu et
86 al., 2003; Smith et al., 2006; Singh et al., 2014). Deterioration can be caused by *Botrytis*
87 *cinerea*, *Alternaria alternata* and soft rots of bacterial origin such as *Pectobacterium*
88 *carotovorum* spp. *carotovorum* (Coplin, 1980; Snowdon, 1991; Fallik et al., 1999;
89 González-Aguilar et al., 1999; Cerkauskas & Brown, 2001; Hadas et al., 2001; Singh et
90 al., 2014). Internal fruit rot caused by FLASC can also be considered as another danger for
91 long term storage of bell pepper (Frans et al., 2017). According to general practices, green
92 peppers should be stored at appropriate temperatures between 7 – 13 °C and high relative
93 humidity (RH) of 95 % to avoid above mentioned quality problems (Polderdijk et al., 1993;
94 Cantwell, 1996; Sharma et al., 2018). Temperatures above 13 °C accelerate fruit ripening
95 and result in a higher susceptibility for pathological deterioration. Colder storage
96 temperatures can cause chilling injury in the bell pepper fruit, yet this is highly maturity
97 dependent (González-Aguilar et al., 2004; Smith et al., 2006; Lim et al., 2007). For mature
98 colored fruit, critical boundaries for acceptable fruit quality for Belgian actions lies between
99 7 and 20 °C although optimal temperatures for long term storage are between 8 – 16 °C
100 for 10 d (Schenk, 2021) yet some cultivars can be even stored for 27 d (Maalekuu et al.,
101 2003). The recommended storage temperature is also supposed to slow down mycelium
102 outgrowth of FLASC as this pathogen has been characterized by a narrow temperature
103 optimum around 25 °C with a strong reduction of mycelial growth *in vitro* at temperatures
104 below 10 °C (Frans et al., 2017). However, cold storage (7-16 °C) of naturally infected
105 fruit with FLASC has previously been reported to be insufficient to inhibit symptom
106 development of IFR (Sauviller et al., 2015).

107 Modified atmosphere packaging (MAP) is commonly used to extend the shelf life of
108 fresh fruit and vegetables, including bell peppers (Manolopoulou et al., 2010; Sahoo et al.,

109 2014; Singh et al., 2014; Sharma et al., 2018). In this technology, a dynamic interaction
110 between the physiological activity of the fruit and the package permeability for gasses,
111 results in an equilibrium atmospheric composition which, if appropriate, brings about an
112 extension of the shelf life of the produce and is sometimes also referred as equilibrium
113 modified atmosphere packaging (EMAP) (Castellanos et al., 2016; Mistriotis et al., 2016;
114 Sandhya, 2010). The effectiveness of EMAP is strongly related to the respiration of the
115 packed fruit, storage conditions such as temperature and relative humidity and the packing
116 material properties such as film permeability and area (Jacxsens et al., 2000; Farber et
117 al., 2003; Mangaraj et al., 2009; Zhang et al., 2015; Castellanos et al., 2016). Typically,
118 low O₂ concentrations (2-5 %) retard ripening and respiration of the bell pepper fruit. On
119 the other hand, high CO₂ levels (> 5 %) can cause browning, skin and calyx discoloration,
120 skin pitting and softening (Meir et al., 1995; Saltveit, 2003; Singh et al., 2014). Peppers
121 should therefore ideally stored under conditions of 2-5 % O₂ and CO₂ levels below 5 %
122 (Polderdijk et al., 1993; Meir et al., 1995; Manolopoulou et al., 2010; Sharma et al., 2018;
123 Devgan et al., 2019). In bell peppers, MAP has already been shown to suppress fungal
124 decay caused by *Botrytis cinerea* and *Alternaria alternata* (Meir et al., 1995; González-
125 Aguilar et al., 2000; Raffo et al., 2007; Manolopoulou et al., 2010; Sharma et al., 2018).
126 On the other hand, the high RH in the bags could favor moisture condensation under
127 fluctuating storage temperatures, thereby creating ideal conditions for growth of soft rot
128 organisms (Polderdijk et al., 1993).

129 The aim of the current study was to evaluate the possibilities of MAP to inhibit FLASC
130 development thereby maintaining bell pepper quality. Bell pepper cultivars with different
131 susceptibility towards IFR (based on greenhouse trials) were selected as they could provide
132 more insight in FLASC proliferation in vivo as resistant cultivars still showed symptom
133 development. Besides the effect on pathological decay by FLASC, a series of important
134 quality parameters for bell pepper fruit quality such as water loss, firmness, color and
135 taste-related parameters such as vitamin C, titratable acidity and total soluble solids were
136 evaluated.

137 2 MATERIALS AND METHODS

138 2.1 *Fruit material and experimental setup*

139 The four bell pepper cultivars used in this experiment (Table 1) were grown in rockwool
140 bags (L x W x H = 120 x 20 x 7.5 cm) in a climate-controlled non-enlightened greenhouse
141 from November 2015 till October 2016, at research stations PSKW and PCH located in the
142 Antwerp province, Belgium. Plants were transplanted half of November and pruned in V-
143 system with 3 stems resulting in a final stem density of 7.2 stems m⁻². Pruning was
144 conducted weekly allowing fruit set on the main stem or in the first node of the side shoot.
145 Plants were fertigated with a standard pepper nutrient solution (Supplementary table 1)
146 with EC 2.5 and pH 5.5 resulting in an EC 3.5 and pH 6.5 in the rockwool slabs. Average
147 greenhouse temperature was 22 °C (25/18 °C, day/night) and RH was set at 75 % with a
148 minimum of 60 % and maximum of 80 %.

149 Prior to the experiments described in this paper, bell pepper cultivars were assessed for
150 their IFR susceptibility by visual inspection of 10 cut open fruit for active IFR symptoms.
151 Inspection was performed every week for 20 w during five consecutive growing seasons
152 (2010-2015). Based on these resistance greenhouse trials, fruit from a sensitive and
153 resistant cultivar for both red and yellow bell peppers were picked during July 2016.
154 Harvesting was conducted at a maturity stage 7 w (yellow) and 8 w (red) after flowering
155 with a maximum of 5 % greenness on the fruit which were immediately transported to the
156 laboratory for analyses and storage treatments. From each cultivar, 30 non-damaged fruit
157 were selected based on their uniformity in size (83 – 95 mm Ø), weight and color. A first
158 group consisting of five fruit of each cultivar and treatment was immediately analyzed
159 (fresh sample) (total n = 10). All other fruit were labelled and randomly divided into two
160 groups and subjected to either storage without packaging (Control) or storage after
161 packaging (MAP). Before inoculation, all fruit were first weighted with ± 0.01 g accuracy
162 (ATL-822, Acculab Atilon, Germany). Using a microsyringe, each fruit placenta was then
163 individually inoculated internally via the blossom end with 0.5 ml of 10⁴ spores ml⁻¹ spore
164 suspension of FLASC type 1 (MUCL 51511). By using this method of inoculation, natural

165 IFR symptoms were mimicked with no external symptoms. Within an hour of inoculation,
 166 fruit from the MAP object were packaged into 60 μ m LDPE pouches (435 cm³ m⁻² h⁻¹ for
 167 CO₂ and 125 cm³ m⁻² h⁻¹ for O₂, Euralpack, Belgium).

168 Using a bench top vacuum sealing machine (C 200, Multivac, Germany) LDPE pouches
 169 were sealed under normal atmospheric conditions. All bell pepper fruit were consequently
 170 stored at 20 \pm 1 °C. This temperature was preferred to create **conducive** conditions for
 171 FLASC growth (worst case scenario) as lower temperatures have been shown to slow down
 172 FLASC growth **but this temperature was still acceptable for fruit quality** (Frans et al., 2017;
 173 **Vanhees et al., 2020**). Five replicates from each treatment were sampled at 7 and 14 d of
 174 storage. At each sampling point, peppers were evaluated for weight loss, internal fruit rot
 175 occurrence, firmness, total soluble solids (TSS) and color change. For each sample, 20 g
 176 of bell pepper were frozen immediately at -80 ° C to allow determinations of ascorbic acid
 177 content and total titratable acidity (TA).

178 **Table 1:** Overview of the 4 bell pepper cultivars used in this experiment and their average IFR percentages based
 179 on 5 growing seasons (2010 -2015) as observed in greenhouse trails. Weekly, 10 fruit were visually checked for
 180 active symptoms of IFR for 20 w per growing season.

PEPPER TYPE	COLOUR	SENSITIVITY	CULTIVAR NAME	ABBREVIATION	IFR (%)
Bell pepper	Red	Sensitive	Redline	RSen	13
<i>C. annuum</i>		Resistant	Redwing	RRes	6
	Yellow	Sensitive	Sensatio	YSen	18
		Resistant	Allrounder	YRes	3

181

182 2.2 Headspace gas evaluation

183 Changes in CO₂ and O₂ concentration (%) of the in-package atmosphere were monitored
 184 on 0, 3, 7, 10, 14 d respectively. A rubber septum (white, Ø 15 mm, PBI Dansensor) was
 185 fixed on the film and pierced with a needle (Ø 0.5 mm) connected to a headspace analyzer

186 (Checkpoint II O₂/CO₂, PBI Dansensor, Denmark). Before each series of readings,
187 calibration of the instrument was performed with O₂ and CO₂ air percentages. At 0 d, initial
188 headspace gas concentrations were measured within an hour after sealing.

189 2.3 *Fruit color determination*

190 Fruit color was evaluated with a Minolta CR-5 Chromameter (Konica Minolta Corp. Japan)
191 operated in the L*a*b* mode as recommended by the Commission Internationale de
192 l'Éclairage (CIE). Color measurements were taken on two opposite sides of each tested
193 sample during every week of storage. The L* variable ranges from 0 (black) to 100 (white)
194 and is a useful indicator for oxidative browning or pigment related changes. The a* scale
195 measures the degree of redness (+a*) and greenness (-a*) while the b* scale measures
196 the degree of yellowness (+b*) and blueness (-b*) (McGuire, 1992; Manolopoulou et al.,
197 2010). The measured a* and b* values were converted into hue angle degrees $Hue =$
198 $\arctan^{-1}(b^*/a^*)$. Hue values of 0 ° correspond to intense red-purple color whilst values
199 close to 90 ° indicate a yellow color (McGuire, 1992). Both values are used as knock-out
200 (KO) criteria by Flanders Centre of Postharvest Technology (VCBT) to determine bell
201 pepper premium quality (FLANDRIA). Red peppers should have L*-values < 36 and hue
202 values < 28 whereas for yellow peppers L* values should be < 59 and hue values < 81 for
203 the quality label (Vanhees et al., 2020).

204 2.4 *Weight loss*

205 Weight loss was calculated as percentage of the initial mass of each sample using
206 laboratory scales with ±0.01 g accuracy (ATL-822, Acculab Atilon, Germany). Weight loss
207 was expressed as the ratio in percentage of weight loss to initial weight (Meir et al., 1995).

$$208 \quad \text{Weight loss (\%)} = \frac{(W_0 - W_t)}{W_0} \times 100$$

209 Where W₀ was the initial weight and W_t was the weight at time t (0, 7 and 14 d).

210 2.5 Firmness

211 Firmness was measured using a Texture Analyzer TA-XT plus (SMS, England). The firmness
212 puncture measurement was conducted with a cylindrical stainless steel probe of 5 mm
213 diameter using a 5 kg load cell. The speed of the probe was set at 1 mm s⁻¹. Puncture tests
214 were carried out on all fruit by pressing the probe vertically against two opposite equatorial
215 sides of the same fruit. Firmness was measured as the average of the values measured on
216 these both sides and expressed in N (de Jesús Ornelas-Paz et al., 2015; Sahoo et al.,
217 2014).

218 2.6 Total Soluble Solids (TSS)

219 Using a garlic press, juice was extracted out of 50 mg of fresh fruit into an Eppendorf tube
220 of 1.5 ml. Total Soluble Solids percentage was determined on 100 µl juice using a ABBE 5
221 hand refractometer (Bellingham & Stanley Ltd., UK) and expressed as %.

222 2.7 Total titratable acidity (TA)

223 Frozen fruit samples were heated for 40 minutes in a 80 % methanol at 80 °C. Total
224 titratable acidity was then determined by titration of methanol extracts against 0.005 M
225 NaOH with phenolphthalein as indicator and expressed as mmol kg⁻¹ fresh weight of the
226 initial fruit mass (Zhang et al., 2020).

227 2.8 Vitamin C content

228 The total content of ascorbic acid was determined using the 2,6 dichlorophenolindophenol
229 method (AOAC, 1990) and expressed as g kg⁻¹ fresh weight of the initial fruit mass.

230 2.9 Internal fruit rot evaluation

231 For assessing internal fruit rot, fruit were cut and evaluated with a numeric, discrete scale:
232 3 = heavy symptoms internal and external on the fruit, 2 = internal mycelium and necrosis
233 on the ovary and inner fruit wall, 1 = restricted mycelium growth on the ovary and 0 = no
234 visible symptoms (Figure 1).



235

236 **Figure 1:** Visualization of internal fruit rot scores for bell pepper after inoculation with FLASC with 0 = no visible
 237 symptoms (A), **circle marking the initial infection scar**, 1 = restricted mycelium growth on the ovary (B), 2 =
 238 mycelium and necrosis on the ovary and inner fruit wall (C) and 3 = heavy symptoms on both in- and outside of
 239 the fruit, **exterior symptoms are visible as sunken lesions** (D)

240 2.10 Statistical analyses

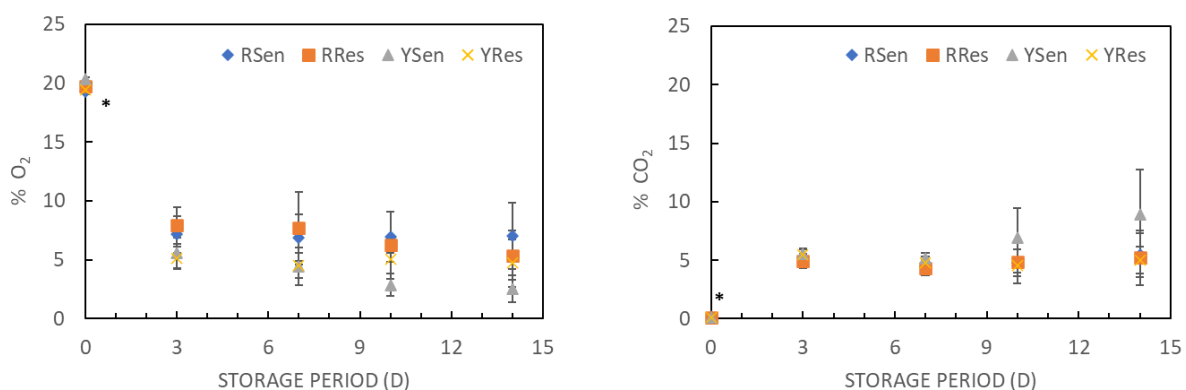
241 Results are expressed as mean \pm standard deviations (SD) of five fruit in a full factorial
 242 design (Cultivar x Storage conditions x Storage time). For statistical analyses, general
 243 linear model (GLM) was conducted on the data using SPSS (IBM Inc., Armonk, NY, USA).
 244 If homogeneity of variances was not fulfilled, a log transformation was conducted on mean
 245 values and GLM was performed with the Weight Least Square (WLS) method. Each
 246 significant factor was analyzed by either analysis of variance (ANOVA) followed by Tukey

247 post hoc test (for Cultivar and Storage time) or independent T-test (Storage Condition)
 248 with significance levels of $P < 0.05$. An non-parametric Mann-Whitney test was used for
 249 the ordinal data of IFR appearance.

250 3 RESULTS:

251 3.1 Headspace gas evaluation in MAP package

252 After packaging, the evolution in headspace gas composition for O_2 and CO_2 during
 253 the storage experiment was registered for MAP packaged fruit (Figure 2). During the early
 254 stages of storage, all cultivars showed a decrease in O_2 concentration in the bags from 20
 255 % to 7 % and 5 % for red cultivars and yellow cultivars respectively. Concomitantly, CO_2
 256 concentrations increased to ~6 % and stabilized. For Ysen a further increase to ~9 %
 257 seemed to occur but due to the big standard deviation it was insignificant.



258

259 **Figure 2:** In package headspace gas composition changes for O_2 and CO_2 for all four bell pepper cultivars (RSen
 260 = Red sensitive, RRes = Red resistant, YSen = Yellow sensitive, YRes = Yellow resistant) during the 14 d of
 261 storage at 20 °C. Data are represented as mean \pm SD of five replicates. Values indicated with * are significantly
 262 different according to Tukey test ($P > 0.05$) for both O_2 and CO_2 conditions.

263

264 3.2 Color evaluation

265 3.2.1 Red cultivars

266 The registered and calculated color parameters (L^* and Hue) of the red cultivars
 267 are displayed in Table 2. Initial lightness values of 36 ± 1 reduced after 14 d for both red
 268 cultivars in the control samples which were indeed visually darker compared with MAP
 269 packed fruit **indicating signs of oxidative browning**. After 14 d, hue values remained stable
 270 in the RSen cultivar under both storage conditions. In the RRes cultivar, hue values
 271 dropped under both conditions, implying that these fruit became more reddish throughout
 272 the experiment. **Based on the KO criteria of VCBT for red bell pepper fruit (Hue < 28, L^***
 273 **< 36), MAP stored red fruit still meet the premium quality for color after 14 d.**

274 **Table 2:** Color values (L^* , and Hue $^\circ$) for the red bell pepper cultivars (RSen = Red sensitive, RRes = Red
 275 resistant) during storage conditions at 20 $^\circ\text{C}$. Data are means of 5 replicates \pm SD.

	STORAGE PERIOD (D)	RSEN		RRES	
		Control	MAP	Control	MAP
L^*	0	36 ± 1 a	36 ± 1 a	35 ± 1 a	37 ± 2 a
	7	35 ± 1 a	36 ± 2 a	37 ± 2 a	37 ± 3 a
	14	33 ± 1 b	36 ± 1 a *	33 ± 1 b	36 ± 2 a *
Hue ($^\circ$)	0	29 ± 2 a	28 ± 1 a	28 ± 2 a	30 ± 3 a
	7	26 ± 1 a	27 ± 2 a	29 ± 2 a	27 ± 1 b
	14	27 ± 5 a	28 ± 1 a	25 ± 1 b	28 ± 2 b

276 A Values in the same row followed by different letters show significant differences among days according
 277 to Tukey test

278 * Values in the same column followed by * show significant differences between storage conditions
 279 according to student T-test

280 3.2.2 Yellow cultivars

281 The color values for both yellow cultivars are summarized in Table 3. The initial
 282 lightness (L^*) values for colored YSen and YRes were average 65 ± 2 and 62 ± 2
 283 respectively **meeting the recommended criteria for the premium quality ($L^* < 59$)**
 284 **(Vanhees et al., 2020). L^* value fluctuated over time and is probably correlated to the**

285 natural variation between the fruit as it occurred in both control and MAP stored fruit. Hue
 286 values however, stayed stable implying an overall steadiness of color for yellow cultivars
 287 during storage with the exception for the control of YSen. To meet premium quality hue
 288 values should be equal or higher than 81 (Vanhees et al., 2020) but none of the objects
 289 fulfilled this criteria during the experiment.

290

291 **Table 3:** Color values (L * and Hue °) of yellow bell pepper cultivars (Ysen = Yellow sensitive, YRes = Yellow
 292 resistant) during storage conditions at 20 °C. Data are means of 5 replicates ± SD.

293

	STORAGE PERIOD (D)	YSEN		YRES	
		Control	MAP	Control	MAP
L*	0	64 ± 1 a	66 ± 2 a	61 ± 1 a	62 ± 2 a
	7	60 ± 2 a	63 ± 1 b	65 ± 2 b	66 ± 3 b
	14	64 ± 4 a	66 ± 2 a	61 ± 2 a	63 ± 2 a
Hue (°)	0	78 ± 2 a	78 ± 2 a	73 ± 2 a	75 ± 3 a
	7	74 ± 2 b	77 ± 2 a	77 ± 2 b	77 ± 2 a
	14	75 ± 2 b	79 ± 2 a	74 ± 1 a	75 ± 2 a

294 a Values in the same row followed by different letters show significant differences among days according
 295 to Tukey test

296 * Values in the same column followed by * show significant differences between storage conditions
 297 according to student T-test

298

299 3.3 Evaluation of physical and chemical characteristics

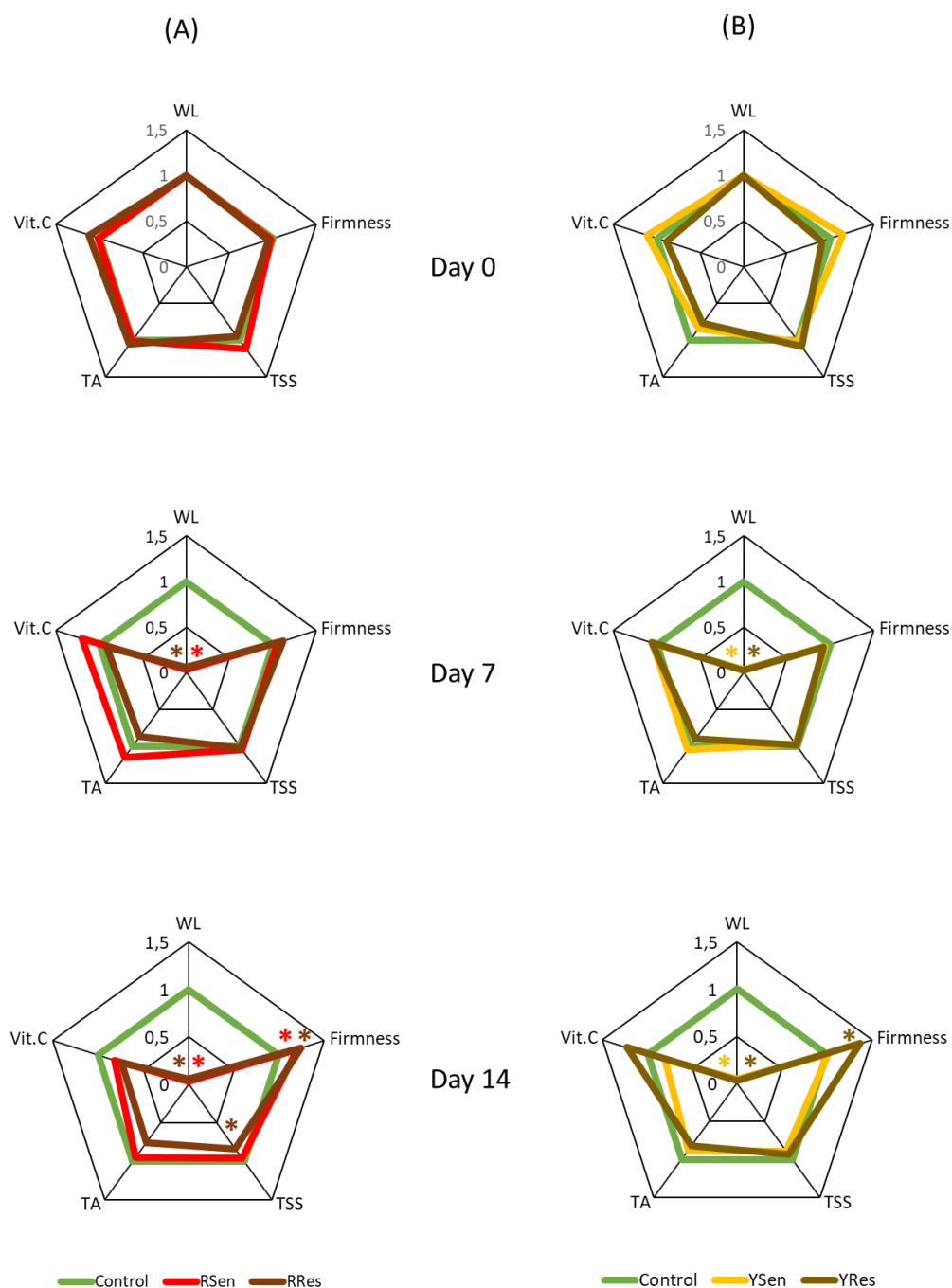
300 As expected, weight loss increased continuously in the control objects with
 301 increasing storage time. After 7 d, control fruit had lost about 5 % of their weight. The
 302 RSen cultivar and YRes showed lowest weight loss (4 % ± 1 for both cultivars) whilst YSen
 303 showed highest weight loss after 7 d (7 % ± 1). At the end of the experiment, similar
 304 results were observed. Weight loss increased after 14 d to an average of 10 % but YSen
 305 showed higher water losses up to 16 % ± 2. In contrast, MAP weight loss was negligible
 306 and restricted to a maximum of about 1 % during the whole experiment (Figure 3)
 307 (Supplementary table 2).

308 The firmness of fresh fruit of all cultivars started around 24 N (Supplementary table
309 3). In the unpackaged, red cultivars, firmness dropped significantly to ~18 N after 14 d.
310 In contradiction, MAP stayed firm in all cultivars (Figure 3 A). Yellow bell peppers were
311 more prone to firmness loss compared to red cultivars. After 7 d, fruit firmness dropped to
312 17 N when stored under atmospheric conditions. MAP could maintain the firmness in less
313 sensitive fruit but was unable to reduce firmness loss in the sensitive cultivar after 14 d
314 (Figure 3 B).

315 TSS started around 5 % and dropped in the RRes cultivar after 14 d. A similar
316 decreasing trend was observed in the other treatments but was not significant
317 (Supplementary table 4) (Figure 3 A).

318 The initial Total Titratable Acidity (TA) for red fruit was about 19 mmol kg⁻¹.
319 Yellow cultivars were characterized by values of 20 ± 3 mmol kg⁻¹ for YSen and 21 ± 5
320 mmol kg⁻¹ for YRes cultivar. During the progress of the experiment, TA did not change in
321 both object for all cultivars. (Figure 3) (Supplementary table 5).

322 Red and yellow cultivars showed similar concentrations of vitamin C (± 1.05 g kg⁻¹).
323 MAP had no influence on vitamin C content in any of the cultivars or treatments (Figure
324 3) (Supplementary table 6).



325

326 **Figure 3:** Spider plots' of weight loss (WL), Firmness, Total Soluble Solids (TSS), Total Titratable Acidity (TA)

327 and Vitamin C (Vit. C) content in bell pepper fruit for control objects (green) and MAP packaged bell pepper fruit

328 of a sensitive cultivar (bright colors) or a resistant cultivar (dark colors) (RSen = Red sensitive, RRes = Red

329 resistant, YSen = Yellow sensitive, YRes = Yellow resistant). Parameters are shown for red cultivars (A) and

330 yellow cultivars (B) and normalized as a percent of their respective control (control = value 1) (n = 5). Asterisks

331 indicate significant differences between control and MAP stored bell pepper fruit at $P < 0.05$ according to the

332 independent T-test.

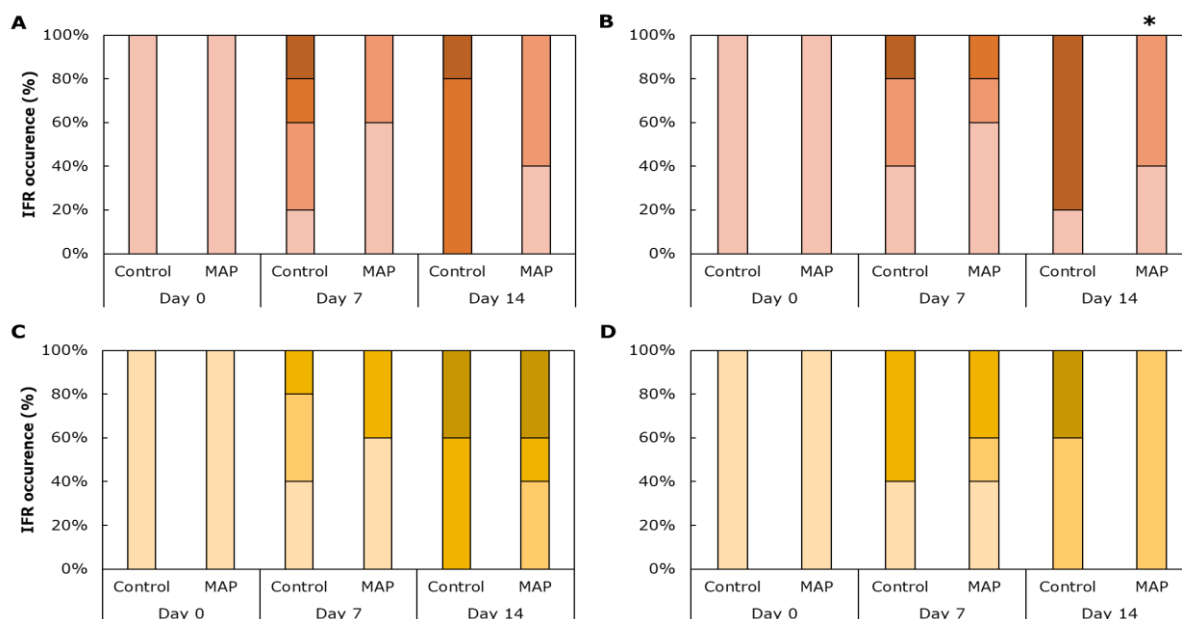
333

334 3.4 IFR symptoms

335 Internal fruit rot symptoms started to appear in all cultivars after the first week of
 336 storage (Figure 4). When stored under MAP conditions, red fruit of RRes showed a **inhibition**
 337 of disease symptoms after 14 d (Figure 4 B). **Similar trends ($P = 0.06$) were observed in**
 338 **the other cultivars (Figure 4A, C and D).** Moreover, a considerable fraction of symptoms in
 339 the MAP treatment of RSen was restricted to either category 0 or 1 (40 %) whilst in the
 340 control treatment, all fruit had symptoms equal or higher than score 2.

341 In the yellow cultivars, a trend ($P = 0.06$) was observed of inhibited fungal
 342 development in MAP YRes fruit. Control objects showed 40 % of the fruit with highest
 343 symptom score (3) whilst no heavy symptoms were detected in MAP fruit. **Ysen fruit**
 344 **showed the least response in disease inhibition.**

345 In addition, no differences in disease severity were observed between the sensitive
 346 and resistant cultivars in both colors.



347
 348 **Figure 4:** Internal fruit rot evolution throughout the storage period for both control and MAP stored bell peppers
 349 of Red sensitive (A), Red resistant (B), Yellow sensitive (C) and Yellow resistant (D) cultivars. Lighter colors
 350 represent lower scores whilst darker colors represent higher scores according to the following scale, 0 = no visible
 351 symptoms; 1 = restricted mycelium growth on the ovary; 2 = mycelium and necrosis on the ovary and inner fruit
 352 wall; 3 = heavy symptoms on both inner- and outside of the fruit (Figure 1). Bars represent the percentage on a
 353 total of 5 fruit. **Bars with an * indicate a significant difference in IFR scores distribution compared to controls.**

354 **4 DISCUSSION:**

355 Internal fruit rot, mainly caused by FLASC, is still a major threat for bell
356 pepper production worldwide and only limited disease treatment and prevention methods
357 are currently available (Utkhede & Mathur, 2005; Frans et al., 2018, 2020). Since, *in vitro*
358 experiments by Frans et al. (2017) showed a significantly slower mycelium growth of
359 FLASC in reduced oxygen conditions, modified atmosphere packaging of fruit was
360 investigated in the current study to potentially diminish FLASC development and reduce
361 economical losses. MAP has already been shown to ameliorate decay by other fungi such
362 as *Botrytis* spp., *Alternaria* spp. and *Penicillium* spp. in bell peppers (Meir et al., 1995;
363 González-Aguilar et al., 2004; Raffo et al., 2007; Manolopoulou et al., 2010). Because the
364 degree of internal fruit rot symptoms has earlier been found to be cultivar dependent (Frans
365 et al., 2016a), two red and two yellow cultivars with different susceptibility for internal fruit
366 rot were selected in the current study.

367 After harvest bell peppers can be stored for two to three weeks at temperatures of
368 7.5-13 °C and a RH of 95-98 % (Gonzalez-Aguilar, 2003). Because earlier *in vitro* studies
369 showed less proliferation of FLASC at lower temperatures (>15 °C), the current study was
370 conducted as a worst-case scenario at 20 °C. Flanders Center for Postharvest Technology
371 stated that quality of bell pepper could be maintained under 20 °C (Schenk, 2021). Even
372 under these favorable temperature conditions for FLASC, low aerobic conditions and
373 elevated CO₂ levels brought by MAP did indeed ameliorate the development and growth of
374 FLASC in fruit of all four bell pepper cultivars. These results clearly corroborate earlier *in*
375 *vitro* work by Frans et al. (2017) and Samapundo et al. (2007) indicate that low storage
376 temperatures in combination with MAP can be attributed to delay activation of the latent
377 fungus and thereby increase shelf life of bell pepper fruits after harvest.

378 In our study no significant differences of disease development were observed
379 between sensitive and resistant cultivars for both colors after artificial fruit inoculation with
380 the pathogen. These observations indicate that sensitivity for IFR is not likely to be caused
381 by different disease development patterns but rather by a diverse susceptibility for initial
382 flower infection under normal growth conditions. Earlier work by Frans et al. (2016b)

383 showed that this susceptibility was not related with flower size and morphology and future
384 research efforts with regard to this topic are encouraged.

385 To determine whether the beneficial effects of MAP were caused by a direct influence
386 on fungal development or a decreased host susceptibility three important physicochemical
387 parameters, i.e. TSS, vitamin C and TA were registered during the experiment. Pathogens
388 are able to adjust their hostile environment in their advantage (Liu et al., 2018). Especially
389 the hosts ambient pH plays a pivotal role in the expression of pathogenicity factors (Prusky
390 et al., 2016; Prusky & Wilson, 2018). Bi et al. (2016) reported that *Fusarium oxysporum*
391 can adjust its ambient pH depending on the amount of carbon sources available in its host.
392 When grown in a liquid medium under carbon excess (e.g. 17 5mM), acidification was
393 induced whereas under carbon deprivation (e.g. 15 mM) alkalization started. In red bell
394 peppers, sucrose levels generally increase during ripening from ± 80 mM to ± 160 mM
395 depending on the cultivar (Hubbard & Mason Pharr, 1992; Aizat et al., 2014). This
396 increasing carbon source could therefore cause an acidification of the bell pepper fruit flesh
397 by the pathogen. In addition, bell peppers are generally characterized by an acidic pH and
398 acidity in peppers increases from pH 6 to 4.5 during ripening (Luning et al., 1994; Estrada
399 et al., 2000; Castro et al., 2005; Castro et al., 2008; Piazzolla et al., 2018). This increase
400 in acidity can then potentially activate the fungus concerning the expression of
401 pathogenicity genes which thrive at low pH (Bi et al., 2016; Prusky et al., 2016) and both
402 FLASC and *F. oxysporum* showed high growth rates at the low pH levels around 3 – 4
403 (Frans et al., 2017). However, both MAP and control fruit did not show significant changes
404 in TSS and TA in our research, which is in accordance with previous controlled packaging
405 studies (Manolopoulou et al., 2010; Rao et al., 2011; Singh et al., 2014). These
406 observations argue in favor of a direct effect from MAP on fungal growth and development
407 rather than influencing host susceptibility. But more detailed work will be required in the
408 near future to further unravel the exact mechanisms behind the fungus-host relationship
409 of FLASC inside bell pepper fruit.

410 Also vitamin C concentrations were not affected by postharvest treatment (MAP).
411 Vitamin C consists of its biologically active form ascorbic acid (AA) and L-dehydroascorbic

412 acid form (DHA) but in bell pepper AA is the major antioxidant relieving oxidative pressure
413 by forming non-oxidative products such as dehydroascorbate and 2,3 diketogulonic acid
414 (Vanderslice et al., 1990; Davey et al., 2000). As such, it can act as neutralizer of Reactive
415 Oxygen Species (ROS) during fungal attack (Kuźniak, 2010) or, in coordination with
416 glutathione, provide the appropriate redox environment for the activation of plant defense
417 responses (Noctor & Foyer, 1998; Boubakri, 2017).

418 As expected, MAP packaged bell pepper fruit suffered less from shriveling due to
419 the high RH inside the bags causing less weight loss and maintaining fruit firmness. In
420 addition, color also remained stable for most cultivars. These positive effects of MAP have
421 been observed in different bell pepper cultivars as well as in spicy *Capsicum* cultivars
422 (Lownds et al., 1994; Manolopoulou et al., 2010; Rao et al., 2011; Chitravathi et al., 2015).
423 On the other hand, the high RH created in the pouches, might accelerate decay of fruit
424 stalks related to *Pectobacterium* spp., which is a common bacterial post-harvest disease
425 of bell pepper (Coplin, 1980; Hadas et al., 2001). Yet this problem can easily be avoided
426 by using moisture absorbers (Singh et al., 2014).

427

428 In conclusion, it can be stated that MAP storage of bell peppers in 60µm LDPE
429 pouches can significantly minimize visible IFR symptoms caused by *Fusarium* spp., even
430 after 14 d in challenging conditions at 20 °C which resembles more unrefrigerated shelf
431 life conditions as in the retail. These conditions are not suitable for normal short or long
432 term storage of bell peppers thus requiring more research under proper storage conditions.
433 A lower temperature (8 °C) experiment with a similar set-up was carried out in a
434 preliminary experiment but no IFR symptoms were observed in all treatments. The lower
435 temperature probably limited FLASC development (Frans et al., 2017). Besides, cold
436 storage of freshly harvested bell pepper fruit which were naturally infected could also not
437 prevent IFR development (Sauviller et al., 2015). Thus, using naturally infected bell pepper
438 fruit and storing them in MAP at low temperatures individually or in bulk crates could
439 further optimize this technique to effectively inhibit IFR development under commercial
440 storage conditions. The use of packages could not only be beneficial for the product quality

441 but could also increase the purchase of bell peppers by consumers by presenting them in
442 attractive combinations (Fernqvist et al., 2015). A classic example of this attractiveness is
443 the flow pack of bell peppers known as the stoplight consisting of a green, yellow and red
444 fruit. Combination of this marketing strategy and MAP could regain consumers trust in
445 buying **high quality** bell peppers.

446

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451

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751

752 **SUPPLEMENTARY TABLES**

753 **Supplementary table 1:** Composition of the standard nutrient solution used for bell pepper production in PSKW
 754 and PCH during the time of experiments in the growing season of 2016.

Macronutrients	mmol/l	Micronutrients	μmol/l
HNO ₃	0.80	CuSO ₄	0.75
NH ₄ NO ₃	0.25	(Na) ₂ MoO ₄ ·2H ₂ O	0.5
KOH	3.30	MnSO ₄ ·H ₂ O	15
Mg(NO ₃) ₂	0.95	ZnSO ₄ ·H ₂ O	5
MgSO ₄	0.85	Na ₂ B ₄ O ₇ ·10H ₂ O	30
H ₃ PO ₄	1.50	Fe-DTPA	25
H ₂ SO ₄	0.90		
Ca(NO ₃) ₂	5.65		

755

756 **Supplementary table 2:** Weight loss (%) of both control and EMPA stored bell peppers of the 4 used cultivars
 757 stored at 20 °C. Fruit under MAP conditions had marginal weight losses whilst control fruit suffered from major
 758 losses. Data are means of 5 replicates ± SD.

Storage period (days)	RSen		RRes		YSen		YRes	
	Control	MAP	Control	MAP	Control	MAP	Control	MAP
0	0	0	0	0	0	0	0	0
7	3.86 ± 0.72	0.12 ± 0.08	4.80 ± 0.33	0.28 ± 0.04	6.64 ± 0.94	0.15 ± 0.03	3.74 ± 0.46	0.07 ± 0.02
14	8.52 ± 1.48	0.17 ± 0.02	11.18 ± 1.09	0.60 ± 0.06	15.80 ± 2.03	0.81 ± 0.34	9.96 ± 0.60	0.32 ± 0.06

759

760 **Supplementary table 3:** Firmness (N) evolution throughout the storage period for both conditions of the 4 used
 761 cultivars. Firmness decreased significantly in all unpackaged fruit during storage period of most cultivars with an
 762 exception for the Ysen. Data are means of 5 replicates ± SD.

Storage period (days)	RSen		RRes		YSen		YRes	
	Control	MAP	Control	MAP	Control	MAP	Control	MAP
0	24.6 ± 3.8	23.9 ± 3.1	24.0 ± 1.9	22.9 ± 2.9	22.8 ± 3.3	26.2 ± 3.4	24.2 ± 3.2	21.7 ± 1.5
7	23.3 ± 2.7	24.7 ± 4.7	21.5 ± 2.4	24.1 ± 2.9	17.3 ± 3.2	16.3 ± 2.7	18.7 ± 3.6	17.2 ± 3.0
14	18.9 ± 3.1	22.7 ± 2.2	16.8 ± 2.4	21.0 ± 2.2	17 ± 2.7	17.2 ± 2.8	17.2 ± 2.9	23.6 ± 2.8

763

764

765 **Supplementary table 4:** Total soluble solids (TSS, %) throughout the storage period. TSS did not fluctuate
 766 severely during the hole experiment. Data are means of 5 replicates \pm SD.

Storage period (days)	RSen		RRes		YSen		YRes	
	Control	MAP	Control	MAP	Control	MAP	Control	MAP
0	5.4 \pm 0.4	6.0 \pm 0.6	5.9 \pm 0.6	5.6 \pm 0.8	5.4 \pm 0.5	5.5 \pm 0.3	4.9 \pm 0.3	5.3 \pm 0.5
7	4.6 \pm 0.6	4.8 \pm 0.2	4.8 \pm 0.3	4.9 \pm 0.5	3.7 \pm 0.5	3.6 \pm 0.6	5.2 \pm 0.3	5.1 \pm 0.5
14	4.8 \pm 0.4	4.6 \pm 0.4	6.1 \pm 0.6	5.1 \pm 0.6	4.5 \pm 0.2	4.0 \pm 0.6	5.5 \pm 0.2	5.1 \pm 0.3

768 **Supplementary table 5:** Total titratable acidity (TA, mmol kg⁻¹) evolution throughout the storage period. TA did
 769 not fluctuate severely during the hole experiment. Data are means of 5 replicates \pm SD.

Storage period (days)	RSen		RRes		YSen		YRes	
	Control	MAP	Control	MAP	Control	MAP	Control	MAP
0	18.7 \pm 5.3	18.9 \pm 2.7	18.1 \pm 2.1	19.1 \pm 3.7	19.5 \pm 2.7	16.5 \pm 5.3	20.8 \pm 4.5	15.9 \pm 3.5
7	19.4 \pm 3.6	22.3 \pm 4.8	18.5 \pm 1.8	16.0 \pm 2.0	15.8 \pm 3.1	16.6 \pm 3.3	17.4 \pm 4.2	15.6 \pm 1.8
14	21.5 \pm 2.3	20.4 \pm 1.9	25.4 \pm 6.9	19.3 \pm 2.5	22.3 \pm 3.8	19.6 \pm 3.7	15.6 \pm 1.8	17.9 \pm 3.2

772 **Supplementary table 6:** Vitamin C (g kg⁻¹) evolution throughout the storage period. Vitamin did not fluctuate
 773 significantly during the experiment when stored under MAP. Data are means of 5 replicates \pm SD.

Storage period (days)	RSen		RRes		YSen		YRes	
	Control	MAP	Control	MAP	Control	MAP	Control	MAP
0	1.03 \pm 0.11	1.04 \pm 0.10	0.97 \pm 0.18	1.08 \pm 0.15	1.04 \pm 0.12	1.15 \pm 0.12	1.20 \pm 0.32	1.07 \pm 0.14
7	1.00 \pm 0.19	1.20 \pm 0.22	1.21 \pm 0.22	1.11 \pm 0.21	0.94 \pm 0.18	0.99 \pm 0.14	0.98 \pm 0.09	1.04 \pm 0.17
14	1.31 \pm 0.17	1.08 \pm 0.18	1.21 \pm 0.21	0.90 \pm 0.17	1.43 \pm 0.24	1.16 \pm 0.26	0.87 \pm 0.26	1.08 \pm 0.24