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

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

1 INTRODUCTION:

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

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

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

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

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

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

2014; Singh et al., 2014; Sharma et al., 2018). In this technology, a dynamic interaction between the physiological activity of the fruit and the package permeability for gasses, results in an equilibrium atmospheric composition which, if appropriate, brings about an extension of the shelf life of the produce and is sometimes also referred as equilibrium modified atmosphere packaging (EMAP) (Castellanos et al., 2016; Mistriotis et al., 2016; Sandhya, 2010). The effectiveness of EMAP is strongly related to the respiration of the packed fruit, storage conditions such as temperature and relative humidity and the packing material properties such as film permeability and area (Jacxsens et al., 2000; Farber et al., 2003; Mangaraj et al., 2009; Zhang et al., 2015; Castellanos et al., 2016). Typically, low O_2 concentrations (2-5 %) retard ripening and respiration of the bell pepper fruit. On the other hand, high CO_2 levels (> 5 %) can cause browning, skin and calyx discoloration, skin pitting and softening (Meir et al., 1995; Saltveit, 2003; Singh et al., 2014). Peppers should therefore ideally stored under conditions of 2-5 % O_2 and CO_2 levels below 5 % (Polderdijk et al., 1993; Meir et al., 1995; Manolopoulou et al., 2010; Sharma et al., 2018; Devgan et al., 2019). In bell peppers, MAP has already been shown to suppress fungal decay caused by *Botrytis cinerea* and *Alternaria alternata* (Meir et al., 1995; González-Aguilar et al., 2000; Raffo et al., 2007; Manolopoulou et al., 2010; Sharma et al., 2018). On the other hand, the high RH in the bags could favor moisture condensation under fluctuating storage temperatures, thereby creating ideal conditions for growth of soft rot organisms (Polderdijk et al., 1993).

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

2 MATERIALS AND METHODS

2.1 Fruit material and experimental setup

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

Prior to the experiments described in this paper, bell pepper cultivars were assessed for their IFR susceptibility by visual inspection of 10 cut open fruit for active IFR symptoms. Inspection was performed every week for 20 w during five consecutive growing seasons (2010-2015). Based on these resistance greenhouse trials, fruit from a sensitive and resistant cultivar for both red and yellow bell peppers were picked during July 2016. Harvesting was conducted at a maturity stage 7 w (yellow) and 8 w (red) after flowering with a maximum of 5 % greenness on the fruit which were immediately transported to the laboratory for analyses and storage treatments. From each cultivar, 30 non-damaged fruit were selected based on their uniformity in size (83 – 95 mm Ø), weight and color. A first group consisting of five fruit of each cultivar and treatment was immediately analyzed (fresh sample) (total n = 10). All other fruit were labelled and randomly divided into two groups and subjected to either storage without packaging (Control) or storage after packaging (MAP). Before inoculation, all fruit were first weighted with ± 0.01 g accuracy (ATL-822, Acculab Atilon, Germany). Using a microsyringe, each fruit placenta was then individually inoculated internally via the blossom end with 0.5 ml of 10^4 spores ml^{-1} spore suspension of FLASC type 1 (MUCL 51511). By using this method of inoculation, natural

IFR symptoms were mimicked with no external symptoms. Within an hour of inoculation, fruit from the MAP object were packaged into 60µm LDPE pouches (435 $cm^3\ m^{-2}\ h^{-1}$ for CO_2 and 125 $cm^3\ m^{-2}\ h^{-1}$ for O_2, Euralpack, Belgium).

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

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

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

2.2 Headspace gas evaluation

Changes in CO_2 and O_2 concentration (%) of the in-package atmosphere were monitored on 0, 3, 7, 10, 14 d respectively. A rubber septum (white, Ø 15 mm, PBI Dansensor) was fixed on the film and pierced with a needle (Ø 0.5 mm) connected to a headspace analyzer

(Checkpoint II O_2/CO_2, PBI Dansensor, Denmark). Before each series of readings, calibration of the instrument was performed with O_2 and CO_2 air percentages. At 0 d, initial headspace gas concentrations were measured within an hour after sealing.

2.3 Fruit color determination

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

2.4 Weight loss

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

$$Weight\ loss\ (\%) = \frac{(W0 - Wt)}{W0} \times 100$$

Where W_0 was the initial weight and W_t was the weight at time t (0, 7 and 14 d).

2.5 Firmness

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

2.6 Total Soluble Solids (TTS)

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

2.7 Total titratable acidity (TA)

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

2.8 Vitamin C content

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

2.9 Internal fruit rot evaluation

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

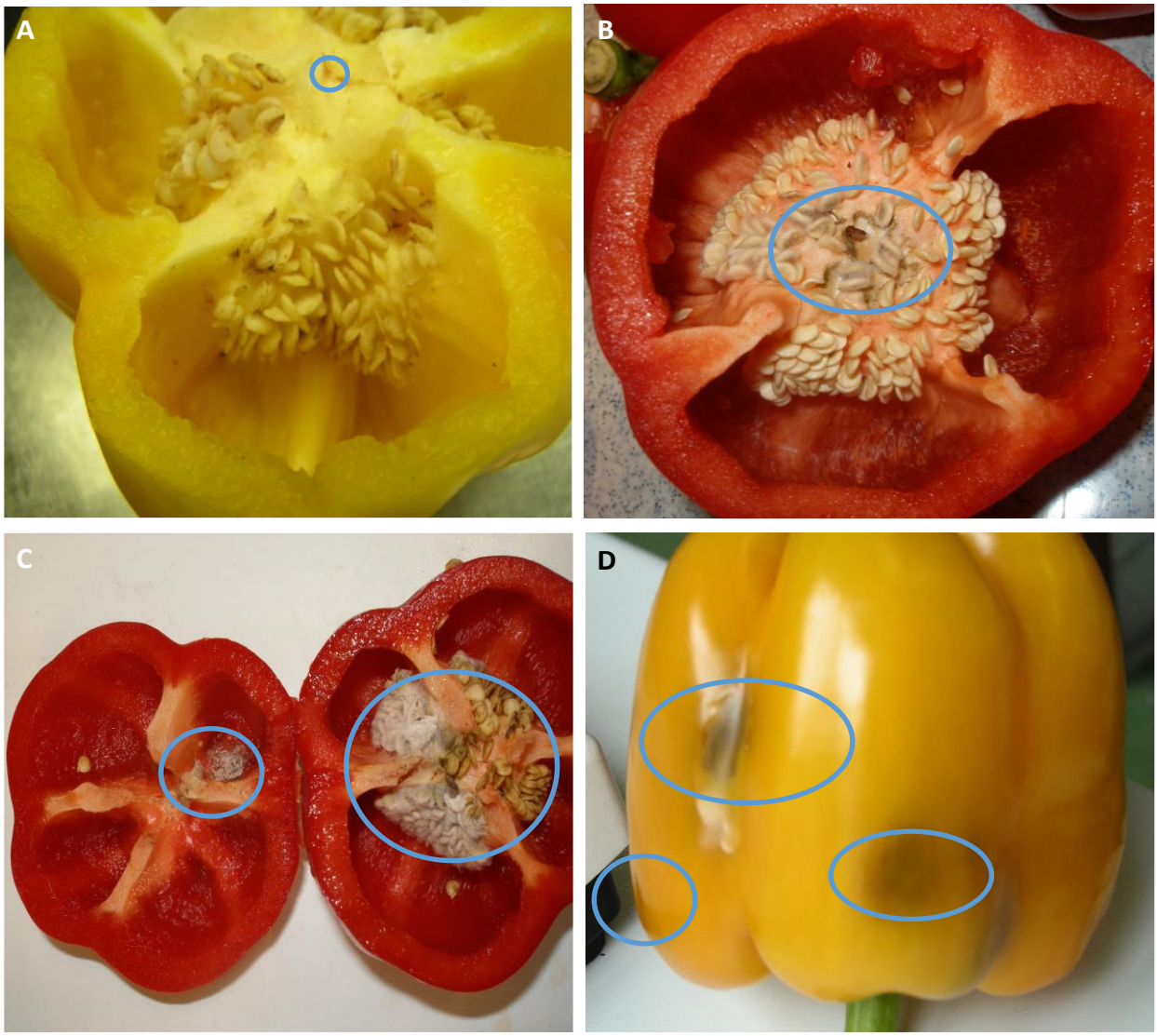

Figure 1: Visualization of internal fruit rot scores for bell pepper after inoculation with FLASC with 0 = no visible symptoms (A), circle marking the initial infection scar, 1 = restricted mycelium growth on the ovary (B), 2 = mycelium and necrosis on the ovary and inner fruit wall (C) and 3 = heavy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

2.10 Statistical analyses

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

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

3 RESULTS:

3.1 Headspace gas evaluation in MAP package

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

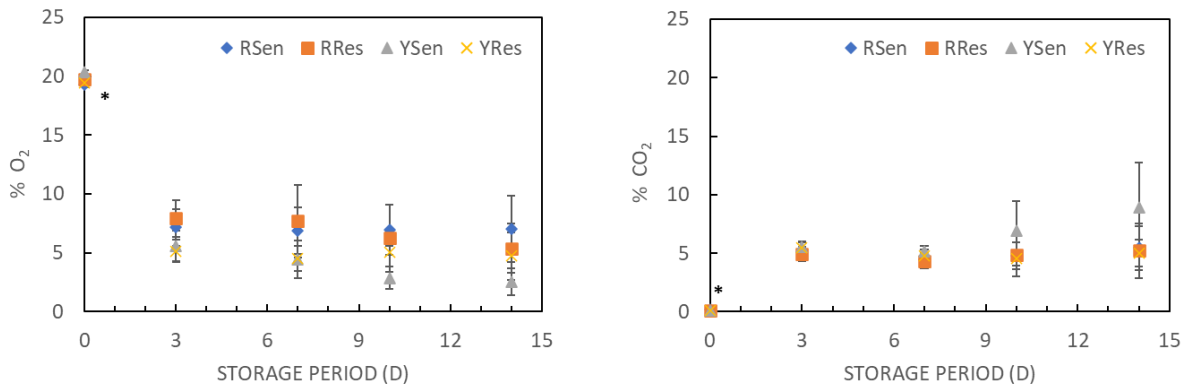

Figure 2: In package headspace gas composition changes for O_2 and CO_2 for all four bell pepper cultivars (RSen = Red sensitive, RRes = Red resistant, YSen = Yellow sensitive, YRes = Yellow resistant) during the 14 d of storage at 20 °C. Data are represented as mean ± SD of five replicates. Values indicated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

3.2 *Color evaluation*

3.2.1 Red cultivars

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

Table 2: Color values (L *, and Hue °) for the red bell pepper cultivars (RSen = Red sensitive, RRes = Red resistant) during storage conditions at 20 °C. Data are means of 5 replicates ± 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 (°)	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

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

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

3.2.2 Yellow cultivars

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

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

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

	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

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

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

3.3 Evaluation of physical and chemical characteristics

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

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

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

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

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

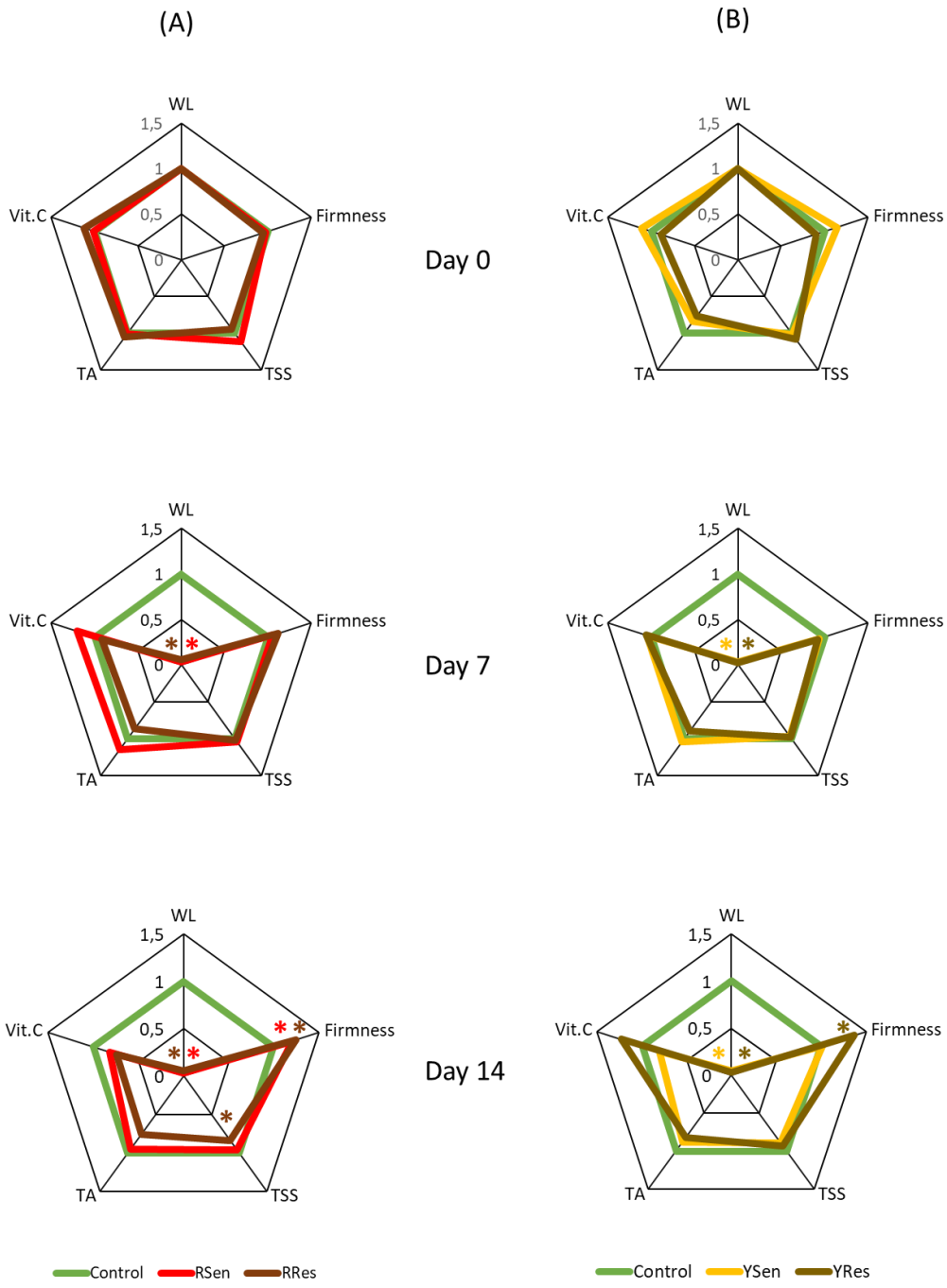

Figure 3: Spider plots' of weight loss (WL), Firmness, Total Soluble Solids (TSS), Total Titratable Acidity (TA) and Vitamin C (Vit. C) content in bell pepper fruit for control objects (green) and MAP packaged bell pepper fruit of a sensitive cultivar (bright colors) or a resistant cultivar (dark colors) (RSen = Red sensitive, RRes = Red resistant, YSen = Yellow sensitive, YRes = Yellow resistant). Parameters are shown for red cultivars (A) and yellow cultivars (B) and normalized as a percent of their respective control (control = value 1) (n = 5). Asterisks indicate significant differences between control and MAP stored bell pepper fruit at $P < 0.05$ according to the independent T-test.

3.4 IFR symtoms

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

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

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

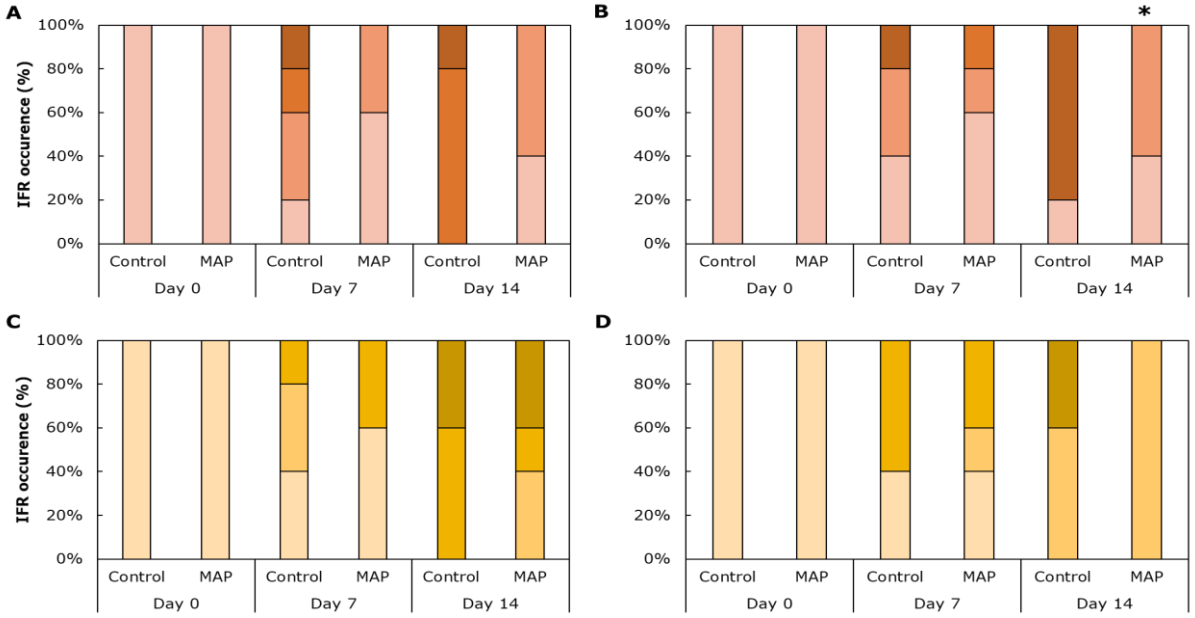

Figure 4: Internal fruit rot evolution throughout the storage period for both control and MAP stored bell peppers of Red sensitive (A), Red resistant (B), Yellow sensitive (C) and Yellow resistant (D) cultivars. Lighter colors represent lower scores whilst darker colors represent higher scores according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

4 DISCUSSION:

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

After harvest bell peppers can be stored for two to three weeks at temperatures of 7.5-13 °C and a RH of 95-98 % (Gonzalez-Aguilar, 2003). Because earlier *in vitro* studies showed less proliferation of FLASC at lower temperatures (>15 °C), the current study was conducted as a worst-case scenario at 20 °C. Flanders Center for Postharvest Technology stated that quality of bell pepper could be maintained under 20 °C (Schenk, 2021). Even under these favorable temperature conditions for FLASC, low aerobic conditions and elevated CO_2 levels brought by MAP did indeed ameliorate the development and growth of FLASC in fruit of all four bell pepper cultivars. These results clearly corroborate earlier *in vitro* work by Frans et al. (2017) and Samapundo et al. (2007) indicate that low storage temperatures in combination with MAP can be attributed to delay activation of the latent fungus and thereby increase shelf life of bell pepper fruits after harvest.

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

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

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

Also vitamin C concentrations were not affected by postharvest treatment (MAP). 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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SUPPLEMENTARY TABLES

Supplementary table 1: Composition of the standard nutrient solution used for bell pepper production in PSKW 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_4NO_3	0.25	$(Na)_2MoO_4.2H_2O$	0.5
KOH	3.30	$MnSO_4.H_2O$	15
$Mg(NO_3)_2$	0.95	$ZnSO_4.H_2O$	5
$MgSO_4$	0.85	$Na_2B_4O_7.10H_2O$	30
H_3PO_4	1.50	Fe-DTPA	25
H_2SO_4	0.90		
$Ca(NO_3)_2$	5.65		

Supplementary table 2: Weight loss (%) of both control and EMPA stored bell peppers of the 4 used cultivars stared at 20 °C. Fruit under MAP conditions had marginal weight losses whilst control fruit suffered from major 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

Supplementary table 3: Firmness (N) evolution throughout the storage period for both conditions of the 4 used cultivars. Firmness decreased significantly in all unpackaged fruit during storage period of most cultivars with an 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

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 ± SD.

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

767

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 ± SD.

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

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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 ± SD.

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

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