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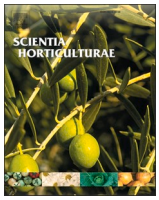

Research Paper

Effectiveness of Sodium Alginate and Carnauba Wax Nanoemulsions with Lemongrass Essential Oil on the quality of 'Hass' Avocado Fruit from early, middle, and late harvest season during prolonged cold storage

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ABSTRACT

Avocado (*Persea Americana* Mill.) has gained popularity as a widely produced and consumed fruit worldwide, raising concerns about its storage and transportation. The avocado, being a climacteric fruit sensitive to chilling injury, faces challenges that affect its shelf life and commercial viability. This research delves into the effectiveness of edible sodium alginate 2 % (weight/weight, w/w) (SA) and carnauba wax 1 % (w/w) (CW) coatings, both independently and in combination with lemongrass essential oil, sodium alginate 2 % (w/w) + lemongrass essential oil 1.25 % (SALG) and carnauba wax 1 % + lemongrass essential oil 1.25 % (w/w) CWLG) as post-harvest treatments. Uncoated avocados were used as control (CT). The nanocoating-treatments aim to preserve the quality of 'Hass' avocados harvested during the early, middle, and late season. After treating the fruits, some from each treatment group were kept at 21 ± 1 ºC for 7 days (shelf life). The rest were stored at 5 ºC and 90 % RH for 15, 30, and 45 days before being submitted also to shelf life. In each sampling date, quality parameters measured included: firmness, color (L*, *hue*), weight loss, gray pulp symptoms, ethylene production and pulp electrolyte leakage. Also, fatty acids (FA), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), α-farnesene (Farn) and conjugated trienols (Ctrols) were quantified in avocado pulp. Fruit quality analysis showed that all coatings improve quality and reduction of gray pulp development, especially CW and the incorporation of lemongrass in both matrices. Therefore, these edible coatings can be an alternative to improve preservation of avocado fruit quality through cold storage and subsequent shelf-life, which potentially reduce fruit waste.

1. Introduction

The avocado (*Persea americana* Mill. Cv. Hass) is a highly sought-after fruit worldwide due to its delicious taste and nutritional benefits (Hashemi and Khaneghah, 2017). Its popularity has made it economically significant, but despite this, the avocado sector still experiences substantial economic losses (Ramírez-Gil et al., 2020). The fruit is vulnerable to mechanical damage, microbial decay, and physiological disorders, leading to high postharvest losses, depending on environmental conditions and inadequate transportation and ripening conditions (Jafarzadeh et al., 2021; Aguirre-Joya et al., 2017; Olivares et al., 2020). As research into health benefits of avocados grows, it becomes increasingly important to investigate factors that affect the quality of the fruit in the market. To extend the shelf life of avocados after harvest, cold storage remains the most common and traditional option, often in conjunction with controlled atmosphere and other novel technologies such as coatings (Gago et al., 2015; Aguirre-Joya et al., 2017; Tesfay et al., 2017; Sierra et al., 2019; Olivares et al., 2020).

Natural coatings that can be consumed are now being acknowledged as a viable, safe, and effective post-harvest solution for preserving fruits.

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These coatings create a protective layer between the fruit and its surroundings, slowing down respiration and altering the gas concentrations, as supported by research (Guerreiro et al., 2015; Hashemi and Khaneghah, 2017; Kumar et al., 2019). Sodium alginate (SA) and carnauba wax (CW), recognized as generally safe (GRAS), serve as key components in these coatings. They form a reliable matrix for trapping bioactive compounds (Otoni et al., 2017) and biocontrol agents (Aloui et al., 2015; Iñiguez-Moreno et al., 2021).

The addition of antimicrobial agents, such as essential oils (EOs), to these coatings further enhances their protective properties against microbial spoilage, thereby prolonging postharvest life and improving overall quality (Antunes et al., 2012). Lemongrass essential oil (LG), extracted from *Cymbopogon citratus* (DC.) Stapf, has demonstrated inhibitory activities against various bacteria and fungi (Gago et al., 2019).

Nanoemulsions offer distinct advantages over conventional emulsions. Their nanometric particle size provides optical clarity, facilitates greater dispersion across cell membranes, optimizes physicochemical and biological properties, and enhances stability and homogeneity (Gago et al., 2019). Edible nanocoatings infused with LG, applied at various concentrations (0.1 %, 0.5 % or 1 %, volume/volume, v/v), demonstrated a remarkable ability to either reduce or completely inhibit the natural microbiota of fresh-cut 'Fuji' apples. This application maintained the firmness of apple flesh nearly intact throughout a two-week storage period (Salvia-Trujillo et al., 2015). A similar formulation, but with a higher concentration of LG (1.25 % weight/-weight, w/w), proved to be effective in preserving the quality of 'Rocha' pear over six months of cold storage and subsequent shelf-life. This approach successfully mitigated superficial scald and delayed ripening (Gago et al., 2020).

Carnauba wax coatings, commonly used to address issues like weight loss, enhance gloss, and control decay in various fruits, have demonstrated additional benefits. They proved effectiveness in reducing chilling injury symptoms in guava and pomegranate (Germano et al., 2019; Nazoori et al., 2023). The incorporation of EOs into CW coatings has further improved their antimicrobial properties (Kubo et al., 2003; Jo et al., 2014). Notably, CW combined with *Cymbopogon martinii* essential oil, when applied to papayas, exhibited potential in delaying ripening, minimizing fresh mass loss, and preventing the emergence of diseases during storage (Oliveira Filho et al., 2022). Moreover, CW contributed to enhancing the visual appeal of the product, imparting shine (Miranda et al., 2022).

In our research, the primary goal was to investigate the impact of nanoemulsions derived from AL and CW, either in their standalone form or enhanced with LG. We aimed to assess their effects on the color, softening, weight loss, ethylene production, α-farnesene (Farn) and conjugated trienols (Ctrols) formation, fatty acids, hydrogen peroxide content (H_2O_2), malondialdehyde content (MDA) and the occurrence of gray pulp chilling disorder in 'Hass' avocado fruit harvested at three different stages (early, middle, and late season) during cold storage plus subsequent shelf life.

2. Material and methods

2.1. Preparation and characterization of nanoemulsions

Nanoemulsions formulation with sodium alginate at 2 % (w/w) (SA) and sodium alginate at 2 % (w/w) + lemongrass essential oil 1.25 % (SALG) were prepared and characterized according to Gago et al. (2020). The nanoemulsions with 1 % (w/w) carnauba wax (CW) were prepared by completely melting the wax at 90 °C and following the procedures described in Gago et al. (2021).

Particle size distribution, polydispersity indexes (PdI), and mean droplet diameters (nm) of nanoemulsions were assessed using a laser diffractometer (Nano-ZS Zetasizer, Malvern Instruments, Worcestershire, U.K.). The measurements were conducted at a wavelength of 633 nm and a temperature of 25 °C, utilizing a backscatter detector (173°). The ζ-potential (mV) of oil droplets in nanoemulsions was determined through phase-analysis light scattering (PALS) using a Zetasizer NanoZS laser diffractometer (Malvern Instruments Ltd, Worcestershire, UK). Prior to analysis, samples were diluted in ultrapure water at a ratio of 1:9 sample-to-solvent (Gago et al., 2019).

2.2. Fruit postharvest treatments

'Hass' avocado were harvested from an orchard in Tavira, southeast Portugal where the protection provided by the mountain barrier against the cold winds from the North and the exposure in an amphitheater facing South, mean that the climate is markedly Mediterranean: hot, dry, not very windy, very low temperature ranges and with an average of insolation above 3000 h of sunshine per year (Rocha, 2018). Fruit were harvested in the same randomly selected trees, for the 3 harvest dates. Harvest was with a 2-month interval, on 2 December 2021, 2 February 2022, and 7 April 2022 (Early, Middle, and Late harvests, respectively). The harvest dates correspond to the start, middle and end of normal harvest period of the producers in the region. Avocados were harvested in the morning and were transported to the University of Algarve. Upon arrival to the lab, in 20 fruits (5 fruits for each of the 4 repetitions) were measured the parameters firmness, color, dry matter content (DMC) and electrolyte leakage in pulp and skin, as described below in point 2.3.

In the harvest day, blemish-free and randomly selected 80 fruits were sprayed with each coating formulation/treatments (SA, SALG, CW, CWLG). Control group (CT) was without any treatment. Sixty fruits (15 fruits/crate and 4 replications) from each treatment were stored in a cold room at 5 °C in normal atmosphere and relative humidity 90–95 % for 45 days. The sampling dates were at harvest with 20 fruits without treatments (5 fruits in each of 4 replications). All the other sampling dates occurred after 7 days of shelf-life at room temperature (22 °C) and 60–65 % relative humidity, following treatment application (without cooling the fruits) and following 15, 30 and 45 days at cold room storage. In each sampling date were evaluated the color (skin and pulp), firmness, weight lost (in shelf-life), pulp electrolytic leakage, ethylene production and symptoms of internal gray pulp. Additionally, fruit pulp samples from each replication/treatment were subjected to drying and freezing for the determination of fatty acids and quantification of Farn, Ctrols, H_2O_2 and MDA.

2.3. Color, electrolyte leakage, dry matter, firmness, and weight loss

Skin color was measured in the CIE L* a* b* color space with a CR-300 colorimeter (CE Minolta, Japan) with a D65 light source and the observer at 10°. The measurements were performed on 3 points of equatorial zone of each fruit, on skin and, after its removal, on the pulp, considering the mean value of the 3 points. The a* and b* readings were converted to the vector coordinate' hue angle (h°) using the equation $h =$ arc tan b* /a* (McGuire, 1992).

Firmness was determined on two opposite sides of each fruit using a Chatillon Force TCD 200 and Digital Force Gauge DFIS 50 (John Chatillon & Sons, Inc., Largo, FL, USA), by measuring the maximum force (N) required for a 6 mm diameter probe (conical for the last 3 mm) to penetrate the avocado fruit to a total depth of 7 mm through the skin and pulp.

Electrolyte leakage (EL) was assessed as previously described (Gago et al., 2016), using an Orion 011,007 conductivity meter (Thermo Scientific Orion StarTM, Beverly, USA). Pulp cylinders with 10 mm diameter and 3–4 mm thickness were used, 5 per replication. The percentage of electrolyte leakage was calculated as a ratio (multiplied by 100) of conductivity measurements before and after boiling. Also, the percentage of dry matter content (DMC) in pulp and skin were calculated as the ratio of the final weight (after dried at 60ºC until constant weight) and initial weight (x100).

Weight loss during shelf life was calculated as percentage of the

initial weight, in samples of 5 fruit from each of the four replications/treatment, after removal from cold room and at the end of the shelf-life period.

Color and firmness measurements were done in 5 fruits per replication, in 4 replicates, as a total of 20 fruits per treatment, and storage condition. Electrolyte leakage and DMC were measured in samples of four replications/treatment, as a composed sample of 5 fruit per replication.

2.4. Symptoms of internal gray pulp

In all sampling dates, all fruits were cut longitudinally in half and the seed removed, so that the entire inner zone of each fruit was visually evaluated for symptoms of internal gray pulp. A total of 20 fruit were observed per treatment and storage time, in 5 fruit per replication.

Additionally, internal fruit physiological disorders and other damages were assessed at all sampling dates using a qualitative scale from 1 to 5: 1 = no occurrence; 2 = slight damage (<25 % damaged area in fruit); 3 = moderate damage (25–49 % damaged area); 4 = moderately severe damage (50–74 % damaged area); 5 = severe damage (>75 % damaged area) (Mendieta et al., 2016).

2.5. Ethylene measurements

'Hass' avocados ethylene production was measured at each harvest time on control fruits and after 15, 30 and 45 days at 5 ºC plus 7 days of shelf-life at 22 ºC, on fruit from all treatments. Two fruits from each replicate (4 replications per treatment) were placed in 1.5 L jars and sealed for 1 h at room temperature (22 ºC). Then, ethylene measurements were performed by withdrawing a 1 mL headspace gas sample from the jars with a syringe and injecting it into a Trace 1300 (Thermo Scientific) gas chromatograph, equipped with a TG-Bond Alumina (Na_2SO_4) 30 m × 0.53 mm × 10 mm (Thermo Scientific) at 60 ºC and a flame ionization detector at 120 ºC. The carrier gas was He at a flow rate of 5.83 × $10^{-7}m^3s^{-1}$. Ethylene production rates were expressed as ng kg^{-1} s^{-1}.

2.6. Extraction and assay of α-farnesene and conjugated trienols

α-Farnesene and conjugated trienols of 'Hass' avocado were extracted from the frozen tissue, of samples composed by 5 fruits of each of the 4 replications/treatment and storage time. Pulp segments (1 g) were extracted with 5 mL hexane (HPLC grade) for 10 min, under constant agitation, at 23 ± 2 ºC. Four separate extractions were performed, one from each replicate. The absorbance of the extracts was read spectrophotometrically at 232 nm and in the range of 281–290 nm (Genesys 10-S, Thermo Electron Corporation, Madison, USA). To calculate the content of α-farnesene (μmol kg^{-1}) and conjugated trienols (μmol kg^{-1}) were used the extinction coefficients ε232nm= 27,740 and ε281–290nm= 25,000, respectively (Guerra et al., 2012).

2.7. Determination of the fatty acid content of avocado pulp

Avocado samples to determine fatty acids were obtained from dried samples composed of 5 fruits for each of 4 replications/treatment. Sample preparation and chromatographic analysis were performed according to the methodology described in Gago et al. (2022).

Additionally, to confirm the identification of fatty acids a sample of avocados was analyzed by Gas Chromatography Time-of-Flight Mass Spectrometry (GC-TOFMS). The GC-TOFMS analysis was performed on Agilent 8890 GC System (Agilent Technologies, UK) coupled to a Bench TOF-Select detector (MARKES International, China) and the data were acquired and analyzed with TOF-DS 4.1 of Markes International. Chromatographic separation was achieved on a Zebron ZB-WAX capillary column (60 m × 0.25 mm i.d. and 0.25 μm df) supplied by Phenomenex (Torrance, CA, USA), using an oven temperature program as described before. Helium was used as carrier gas. The MS transfer line and source temperatures were set at 250 °C. To determine the retention times and characteristic mass fragments, electron ionization (EI) at 70 eV mass spectra of the analytes were recorded at full scan, from 30 to 400 Da. The Supelco™ 37 Component FAME Mix (Mixture of fatty acid methyl esters) was also analyzed using the same chromatographic conditions. The fatty acids were identified by matching mass spectra with spectra of reference compounds in NIST mass spectral library (NIST MS Search Program Version 2020), taking into consideration structure and molecular weight, and by comparison with the retention times of FAs in a standard mixture.

A mixture of fatty acids was used as an external standard. Individual fatty acids were identified and measured by comparing their retention times and peak areas to standards. Results were expressed as percentage of each fatty acid in the total fatty acids identified. The unsaturated/saturated fatty acid ratio was calculated by the formula: sum of unsaturated/saturated fatty acids (UFA/SFA).

2.8. Hydrogen peroxide content and lipid peroxidation

Hydrogen peroxide content and lipid peroxidation products were quantified from the frozen composed samples (4 replications/treatment). Lipid peroxidation was determined through the malondialdehyde method. H_2O_2 content was measured using the method described by Velikova et al. (2000) with some modifications: 0.3 g of fruit pulp samples underwent homogenization with 1.5 mL of 0.1 % trichloroacetic acid (TCA). The resulting homogenate was then centrifuged at 12, 000 rpm for 15 min at 4 ºC. Then, 50 μL supernatant from each sample were placed into a microplate wells, added 50 μL phosphate buffer (10 mM, pH 7.0) and 100 μL potassium iodide (1 M). The absorbance was measured at 390 nm in a microplate reader (Synergy HTX, Biotek, USA). The H_2O_2 concentration was determined by referencing to a standard curve and expressed as μmol/g of fresh weight (FW).

The malondialdehyde content was determined according with Ngoc et al. (2022), using the Genesys 10-S (Thermo Electron Corporation, Madison, USA) spectrophotometer and expressed as nmol/g.

2.9. Statistical analysis

Data analysis was performed using IBM SPSS Statistic 27.0 software (IBM SPSS Inc., NY, USA) on an PC workstation (Intel(R) Core (TM) i7. The experimental design was a complete randomized design. A three-way factorial analysis of variance (ANOVA) was carried out to test the significance of the effects of treatment (SA, SALG, CW, CWLG and CT), storage time (15, 30 and 45 d), harvest date (early, middle, and late), and their interaction. Duncan's multiple-range test ($p < 0.05$) was used for comparing the means. Using the statistical software Chemoface version 1.5 (Nunes et al., 2012), principal component analysis (PCA) was conducted to identify clusters of edible coatings exhibiting comparable effects on the studied parameters.

3. Results and discussion

3.1. Nanoemulsion characterization

All the formulations being examined fall within the nano range (< 500 nm) due to their droplet sizes ranging from 24.23 nm to 179.13 nm (Table 1). The polydispersity values, smaller in CW nanoemulsions, point to a higher uniformity in droplet size. Conversely, values close to 1 imply a heterogeneous distribution (McClements et al., 2012). Particles with zeta potential values exceeding (+/-) 30 mV are generally deemed stable, as the strong electrical charge of droplets implies that repulsive forces between them dominate in the nanoemulsions (Salvia-Trujillo et al., 2015). In our scenario, nanoemulsions with CW alone as the base displayed elevated zeta potential values. However, when these nanoemulsions included EOs, they exhibited higher polydispersity, and zeta

Table 1
Droplet size (nm), polydispersity index (PDI) and zeta-potential (mV) of sodium alginate-nanoemulsions containing lemongrass essential oil (SALG) and carnauba wax-nanoemulsions alone (CW) and with lemongrass essential oil (CWLG).

Nanoemulsion	Droplet size (nm)	Polydispersity index	Zeta-potential (mV)
SALG	24.23±1.19[c]	0.44±0.05[a]	−31.73±8.07[b]
CW	133.87±0.55[b]	0.20±0.01[b]	−29.6 ± 0.69[b]
CWLG	179.13±0.99[a]	0.39±0.01[a]	−16.00±0.3[a]

[a,b,c] Means in same column with different letters are significantly different at $p < 0.05$. Data shown are the means ± standard error. *SA alone is not an emulsion.

potential values. The droplet size in tested nanoemulsions containing CW with values 133.87–179.13 nm closely resembles that achieved by Gago et al. (2021), with values 148.3–163.9 nm, albeit with reduced polydispersity and zeta potential, what makes them more homogeneous and stable than that of the previous authors. Nevertheless, the CW-nanoemulsions employed in this study exhibited larger droplet sizes, increased polydispersity, and higher zeta potential values compared to those documented by Jo et al. (2014) and Ohashi et al. (2015). Additionally, alginate-based nanoemulsions had smaller droplet sizes, but slightly higher polydispersity in comparison to the results reported by Gago et al. (2020).

3.2. Effect of harvest date on fruit characteristics and maturity indices

Lightness (L*) of fruit skin increased only from middle to late harvest, while hue values had the opposite pattern (Table 2). These results are partially contrary to those reported by Arpaia et al. (2018) in which fruit harvested later in the season had lower L and hue values. However, L values measured in this work are higher than those reported by Aguirre-Joya et al. (2017) (L values < 32), possibly due to the difference in pre-harvest or edaphoclimatic conditions. In pulp, hue values were similar in all harvest dates and pulp lightness had the highest value in the fruit of the late harvest.

Fruit firmness was lower in the early harvest (Table 2). This result was unexpected, but since firmness is measured by piercing the skin and pulp of the fruit, the lower firmness of the fruit in the first harvest may be due to less lignification of fruit skin. Effectively, according to Medina-Carrillo et al. (2017) throughout fruit development there is increase in lignin content in the skin.

The skin and pulp DMC increased significantly from early to late harvest (Table 2). DMC is the result of the accumulation of carbohydrates, including structural carbohydrates like fiber, starches, sugars, proteins, vitamins, minerals, lipids, and volatile compounds, (Iñiguez-Moreno et al., 2021), which may lead to higher nutritional quality of the late harvestd fruit.

Table 2
Quality variables at three harvest times in "Hass" avocado fruit.

Fruit characteristics at harvest	Early harvest	Middle harvest	Late harvest
Skin DMC (%)	24.32 ± 0.74**b**	25.54 ± 0.21**a**	26.44 ± 0.8**a**
Pulp DMC (%)	22.24 ± 0.51**c**	27.13 ± 1.00**b**	35.66 ± 0.60**a**
Firmness (N)	138.0 ± 5.54**b**	154.6 ± 3.62**a**	148.9 ± 3.4**a**
Skin color L*	37.32 ± 1.33**b**	37.44 ± 0.74**b**	39.49 ± 1.07**a**
°Hue	128.10 ± 1.57**a**	126.83 ± 0.61**a**	119.63 ± 1.79**b**
Pulp color L*	65.12 ± 1.01**b**	62.72 ± 2.03**c**	70.40 ± 0.78**a**
°Hue	114.98 ± 0.62**a**	114.33 ± 0.69**a**	115.61 ± 6.60**a**
Skin electr. leakage	3.00 ± 0.47**a**	3.44 ± 0.53**a**	3.50 ± 0.32**a**
Pulp electr. leakage	5.81 ± 0.74**b**	7.89 ± 0.52**ab**	8.57 ± 1.3**a**

Values are means of 4 replications ± standard error (SE). Values followed by the same lower-case letter, in the same row and parameter, are not significantly different by Duncan's multiple range test, at $p < 0.05$. DMC = dry matter content.

Electrolyte leakage can be taken as a storage quality parameter, with low values indicating lower membrane permeability (Woolf et al., 2003). In this study, there was a rise in electrolyte leakage from both the skin and pulp between the early and late harvest dates. However, noteworthy differences were observed only in pulp electrolyte leakage, where the values in the late harvest were significantly higher compared to those of the early harvest.

3.3. Ethylene production

Ethylene plays a role in influencing various quality aspects of ripening avocados, such as pulp softening and skin color, which are crucial for determining their storage and commercial potential (Olivares et al., 2020). Ethylene production was not detected at the harvest day (early, middle, or late season) as expected, since avocado does not produce ethylene while attached to the plant (Arpaia et al., 2018).

Ethylene production was clearly affected by harvest date ($p < 0.001$) and cold storage time ($p < 0.001$) with a significant 2-way interaction between these factors ($p < 0.001$). In shelf-life just after harvest, there was no ethylene production in early harvested fruit, there was a low production in the middle harvested and a burst in ethylene production in the late harvest (Fig. 1A), indicating the natural advancing of climacteric fruit maturation (Olivares et al., 2020). Nevertheless, when fruit were subjected to cold storage and posterior shelf life at room temperature, there were no differences in ethylene production among the 3 harvest dates, being the values low (Fig. 1A).

The ethylene production was affected by treatments ($p < 0.01$), with higher values in uncoated than coated fruit and without significant differences among coated treatments, despite a slight lower production in CWLG-coated fruit (Fig.1B). In this work, coating of the fruit reduces ethylene production, increasing storage capacity as previously reported for other fruit (Gago et al., 2022). This happens either due to ethylene production regulation by the film barrier created on the avocado skin, which creates a modified atmosphere within the fruit, or barrier to external ethylene action (Jafarzadeh et al., 2021).

3.4. Ripening behavior through storage time plus shelf-life

3.4.1. Skin and pulp color

Changes in skin color are one indicator of 'Hass' avocado fruit ripening which is usually green at harvest and shifts to purple/black while completely ripe (Arancibia-Guerra et al., 2022). The evaluation of avocado skin color was based on the CIE Lab parameters: lightness and °hue (McGuire, 1992). According to our results, L* values decreased through storage in all treatments, meaning a darkening of the skin, as expected. The decrease was more pronounced after 15d cold storage plus 7 days shelf life.

Nevertheless, there is a tendency to the coatings with SA and SALG to have the higher, closer to harvest values, mainly at the end of storage, for all harvest dates, meaning better effect in delaying color changes. Control showed the lower L* values.

The °hue showed a similar pattern, but here the effect of coatings on reducing color changes is more visible, showing control fruit the lower hue values, mainly at the end of storage, corresponding to more purple/black skin color in all harvest dates. When looking at the first 2 harvest dates (early, middle), the effect of coating is more significant in maintaining flesh green color, with CWLG showing the best results. The effect of coating is reduced in the late harvest date with no significant differences among treatments, at the end of storage. According to Osuna-García et al. (2011), blackening of 'Hass' avocado peel is not linked to diminished fruit quality but serves as an indicator of lower pulp firmness and advanced ripening stage.

There was no significant effect of coatings on avocado flesh color, except in the late harvest (Table 3). In the late harvest, from 30 d cold storage + 7d shelf life, control fruit showed significantly lower °hue

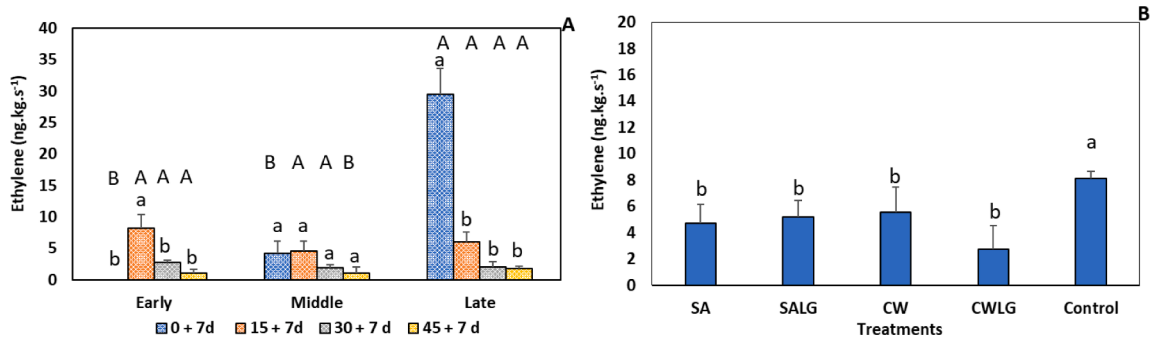

Fig. 1. **(A)** Effect of harvest (early, middle, and late) and cold storage time (0 d (at harvest), 15 d, 30 d and 45 d) in ethylene production, measured after additional 7d shelf life at 22 ºC. **(B)** Effect of nanocoatings in ethylene production of 'Hass' avocado fruit; coatings/nanocoating's contain alginate 2 % (w/w) (SA), sodium alginate 2 % (w/w) + lemongrass essential oil 1.25 % (w/ w) (SALG), carnauba wax 1 % (w/w) (CW), carnauba wax 1 % (w/w) + lemongrass essential oil 1.25% (w/ w) (CWLG) and control (fruit as it comes from the field). Values are means ± standard error. Bars with different lower case in the same harvest, and the same bar-sampling date with the same upper case are not significantly different by Duncan's multiple range test, at $p < 0.05$.

values in the pulp, corresponding to a higher pulp ripening. As suggested by Maftoonazad & Ramaswamy (2008), the reduced color changes in coated fruits could be attributed to the coating's influence on modifying the atmosphere within the fruit, since higher CO_2 levels in storage atmosphere play a crucial role in preventing chlorophyll degradation (Li et al., 2019).

3.4.2. *Fruit firmness and pulp electrolyte leakage*

The decline in firmness is linked to the breakdown of polysaccharides through enzyme action, such as pectinases, cellulases, amylases and polygalacturonases (PG). These enzymes induce structural changes in the cell wall, resulting in fruit softening (Pinto et al., 2019; Shezi et al., 2020). In this work, avocado firmness was affected by treatment ($p < 0.001$), cold storage time ($p < 0.001$) and harvest date ($p < 0.001$), with a significant 3-way interaction among these factors ($p < 0.001$). When ripened under shelf-life conditions without previous storage, CT fruits exhibited lower firmness, particularly in middle and late-harvested fruits compared to early-harvest ones (Fig. 2A, 2B, 2C).

In all harvest, just 15 d of cold storage induced a significant decrease in fruit firmness, these tendencies being similar for coated and control fruits, showing the effect of chilling in triggering ripening in some fruits (Zauberman et al., 1985) (Fig. 2A, B, C). This trend was decreased with later harvest dates, due to advanced maturity stage of the fruit, with lower firmness at harvest, as reported by Mpai and Sivakumar (2020). In fact, CT fruits, at late harvest, not submitted to cold storage or submitted to different cold periods, showed similar firmness values in shelf life (Fig. 2C). Nevertheless, till 15 days plus 7 d shelf life, fruit coated with CW showed slightly higher firmness values, what may be an important factor while transporting through the seas, the avocado fruit for the market, or even when fruit is sent immediately to the market.

3.4.3. *Pulp electrolyte leakage*

Electrolyte leakage of plant tissue measures cell membrane integrity and can be a significant indicator of the chilling susceptibility (Hershkovitz et al., 2009). These authors reported that electrolyte leakage increased in 'Arad' and 'Ettinger' avocado fruit stored at 5.0 °C and this correlated with the manifestation of chilling injury symptoms when held to ripen at 20 ºC.

Globally, pulp electrolyte leakage was affected by treatment ($p < 0.001$) and cold storage time ($p < 0.001$) but was not affected by harvest date ($p = 0.197$). However, these 3 factors interact and interfered with electrolyte values ($p < 0.001$), therefore each factor was ultimately analyzed at fixed levels of the two other factors. Before storage, the pulp electrolyte leakage values increased from early to late harvests in all treatments, and in the two last harvests the CT fruits showed the highest values after 7 days of shelf-life storage.

In all harvests, just 15 days of cold storage induced a significant increase in pulp electrolyte leakage, these trends were similar for coated and CT fruit, although the rate of increase was lower in the late harvest (Fig. 2D, E, F). This observation confirms the effect of chilling in advancing the ripening process as confirmed by firmness measurements (Zauberman et al. (1985).

In fact, coatings were efficient in reducing electrolyte leakage in middle and late harvested avocado fruits ripened without cooling storage, and in the early harvested fruit subjected to 15 days storage (Fig. 2D, E, F). Due to the correlation found between chilling injury and electrolyte leakage, the coatings may play a role in reducing those disorders (Hershkovitz et al., 2009).

3.4.4. *Weight loss*

Weight loss is an important postharvest quality criterion for avocado once it results in loss of quality and freshness and subsequently implies economic loss. Shelf-life weight loss was affected by harvest date ($p < 0.001$), cold storage period ($p < 0.001$) and treatment ($p < 0.001$), also by two significant 2-way interactions harvest x treatment ($p < 0.001$) and harvest x cold storage period ($p < 0.001$).

The late harvest had a pattern of weight loss in the shelf-life that differed from the two previous harvests (Fig. 3A). The fruits had minimal weight loss in the shelf-life that followed the harvest but exhibited greater weight loss in the shelf-life after prolonged cold storage. Therefore, in the late harvest, and particularly in the shelf-life following the 30 and 45 days of cold storage, the weight loss of the fruits reached the highest values, which may be related to the greater permeability of fruit skin (Fig. 3A) which would have allowed a greater migration of water to the outside of the fruit.

It is clear that in the late harvest, control was the one with higher weight loss, showing the important effect of coating in avocado late harvest (Fig. 3B). Nevertheless, in early and middle harvest, control and SA had a slight tendency to have more weight loss. This was also shown in the last harvest, although without significant differences, making the CW coating the most efficient to reduce weight loss. This may be due to the coating composition since SA is hydrophilic and CW is hydrophobic due to its lipidic nature (Aloui et al., 2015; Miranda et al., 2020).

3.5. *Gray pulp*

Gray pulp is a dispersed area of discolored gray or gray/brown of fruit mesocarp without delimited margin, usually starting at the bottom of the fruit adjacent to the seed and spreading to all mesocarp tissue which appears after prolonged cold storage (Olivares et al., 2020).

The data showed that the intensity of gray pulp symptoms in fruits was affected by harvest ($p < 0.001$), cold storage time ($p < 0.001$) and treatment ($p < 0.001$), and interaction between harvest date and cold storage time ($p < 0.001$). In the three-harvest dates, the initial symptoms

Table 3
Color parameter (luminosity, and tonality), in "Hass" avocado fruit coated with sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT), harvested in early, middle, and late in the season. Sampling dates were just after post-harvest treatment and after 15, 30 and 45 days of cold storage plus 7d shelf-life.

Color parameter		Treatment	Skin				Pulp			
			At harvest	15d cold	30d cold	45d cold	At harvest	15d cold	30d cold	45d cold
L*	Early	SA	36.80 ±0.27aA	33.54±0.52aB	33.59±0.25aB	31.72 ±0.25aC	65.85 ±1.14abA	64.495 ±1.03aA	64.07±0.5abA	64.82 ±0.31aA
		SALG	35.55 ±0.28bA	32.49±0.26aB	32.24 ±0.43bBC	31.18 ±0.24abC	64.38 ±0.55abA	64.37±0.52aA	65.16±0.42cA	64.60 ±0.66aA
		CW	35.08 ±0.12bA	33.68 ±0.97aAB	32.24 ±0.37bBC	30.48 ±0.42bcC	64.85 ±0.87abA	65.439 ±0.42aA	65.16±0.41aA	64.92 ±0.88aA
		CWLG	34.86 ±0.32bA	33.07±0.86aB	32.00±0.13bB	31.94 ±0.28aB	63.97±0.14bA	64.07±0.81aA	63.78 ±0.72abA	65.10 ±0.06aA
		CT	36.60 ±0.24aA	33.07±0.71aB	32.35±0.19bB	29.88 ±0.32cC	66.54±0.6aA	65.63±0.84aA	64.73±0.38aA	61.69±0.8bB
	Middle	SA	37.54 ±0.29aA	32.23 ±0.36abB	30.99 ±0.44abC	31.80 ±0.33bBC	65.93±0.45aA	66.08 ±0.75abA	65.47±0.44aA	62.14 ±0.43cB
		SALG	36.65±0.1bA	32.96±0.49aB	29.90±0.5abC	33.35 ±0.24aB	66.94±1.57aA	67.40±0.45aA	65.42±0.17aA	65.98±1abA
		CW	35.89 ±0.31bcA	31.03 ±0.69bcB	29.90±0.29bB	30.56 ±0.42cB	65.23±0.38aA	65.63 ±0.97abA	65.42±0.83aA	65.51 ±0.87abA
		CWLG	35.54 ±0.23cA	32.21±0.5abB	31.39 ±0.22aBC	31.01 ±0.16bcC	67.72±0.45aA	65.56 ±0.45abAB	65.88 ±0.75aAB	64.60 ±0.95bB
		CT	35.70 ±0.26bA	30.36±0.69cB	31.32±0.49aB	30.73 ±0.17cB	66.79±0.7aA	65.075 ±0.39bB	65.43±0.77aB	67.42 ±0.47aA
	Late	SA	37.60 ±0.57abA	30.65±0.48aB	30.21±0.35aB	30.16±0.4aB	67.82±0.57bA	69.06±0.83aA	69.21±0.61aA	64.44 ±1.22aB
		SALG	38.87 ±0.44aA	30.35±0.76aB	28.32 ±0.24bC	31.18 ±0.27aB	70.79±0.34aA	70.88±0.23aA	70.12±0.39aA	66.23±2aB
		CW	36.04 ±0.87bcA	30.66±0.43aB	28.32 ±0.22bC	28.00 ±0.41bC	70.31±0.4aA	70.23±0.79aA	70.12±0.7aA	65.78 ±0.09aB
		CWLG	37.05 ±0.87abA	29.90±0.64aB	28.55±0.43bB	28.84 ±0.32bB	69.09±0.8abA	70.91±0.32aA	70.61±0.4aA	64.49 ±1.27aB
		CT	34.81±0.7cA	29.99±0.56aB	28.42±0.28bB	28.60 ±0.33bB	69.57 ±0.63abA	70.76±0.37aA	69.07±1.34aA	58.49 ±2.10bB
°Hue	Early	SA	126.99 ±0.38aA	93.04±5.77cC	109.81 ±0.67bB	91.68 ±1.43cC	113.88 ±0.32aB	113.47 ±0.51aB	116.26 ±0.23abA	113.73 ±0.58aB
		SALG	127.44 ±0.46aA	107.42 ±3.77bB	108.03 ±1.08bcB	89.99 ±1.73cC	114.21 ±0.27aBC	114.59 ±0.57aB	118.15 ±0.09aA	113.38 ±0.39aC
		CW	127.62 ±0.22aA	114.70 ±2.24abB	106.51 ±0.52cC	102.47 ±1.91bC	114.07 ±0.28aB	114.34 ±0.26aB	118.15±2.2aA	113.06 ±0.59aB
		CWLG	128.04 ±0.04aA	119.43 ±3.01aB	113.75 ±0.79aC	110.06 ±0.96aC	114.18 ±0.11aB	114.75 ±0.24bAB	115.25 ±0.5abA	114.26 ±0.17aB
		CT	127.20 ±0.33aA	91.61±2.13cC	103.29 ±1.05dB	83.61 ±1.33dD	113.82 ±0.23aAB	114.66 ±0.54bA	114.05 ±0.23bAB	112.97 ±0.24aB
	Middle	SA	126.15 ±0.25aA	82.38 ±2.62bcC	88.75 ±6.57aBC	94.49 ±1.91cB	114.63 ±0.29aA	114.54 ±0.34abA	115.19±0.1aA	114.10 ±0.13aA
		SALG	127.42 ±0.36aA	89.68 ±3.85abC	87.67±1.78aC	101.92 ±1.84bB	113.48 ±0.6abA	113.66 ±0.58aA	115.54 ±4.87bA	115.27 ±0.34aA
		CW	127.44 ±0.3aA	89.56 ±8.83abA	87.47 ±2.55aA	99.18 ±1.65bcA	114.33±0.2aA	114.61 ±0.47abA	115.54 ±0.55aA	115.09 ±0.29aA
		CWLG	126.99 ±0.39aA	107.27 ±2.81aB	98.54±1.1aC	106.93 ±1.36aB	112.75 ±0.25bC	114.26 ±0.17aAB	114.75 ±0.45abA	113.69 ±0.27bB
		CT	114.05 ±1.45bA	67.31±9.64cC	90.70±5.92aB	97.71 ±0.93bcB	113.84 ±0.32abB	114.49 ±0.27aAB	114.94 ±0.37abA	113.81 ±0.25bB
	Late	SA	106.40 ±3.93aA	42.52±3.81bC	61.46±2.22aB	63.12 ±1.53aB	114.15 ±0.36aA	112.67 ±0.61aA	112.11 ±0.46aA	106.65 ±0.66aB
		SALG	111.12 ±4.23aA	38.04±8.93bC	41.01 ±2.24bC	61.70 ±1.54aB	112.66 ±0.17aA	113.16 ±0.11aA	111.68 ±0.25aA	107.85 ±1.12aB
		CW	107.10 ±3.16aA	65.85±2.89aB	56.29±5.11aB	59.05 ±3.73aB	112.67 ±0.16aA	112.93±0.3aA	111.68 ±0.58aA	108.28 ±0.82aB
		CWLG	113.42 ±2.52aA	69.84±4.17aB	56.52±5.21aC	67.07 ±2.72aBC	113.02 ±0.35aA	112.75 ±0.18aA	112.46 ±0.16aA	110.48 ±3.14aB

Values are means ± standard error. Values in the same column followed by different lower-case and in the same row followed by different upper-case, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

of gray pulp appeared only after 30 days of storage plus shelf life and increased with the prolongation of cold storage. Therefore, the analysis was carried out considering only the 30 and 45 days of cold storage. The symptoms also increased from the early to the late harvest.

It is visible that as later is the harvest time, more susceptible are the fruit to gray pulp development through storage (Fig. 4A). Fruits coated with CWLG and CW alone exhibited the least severity of gray pulp symptoms, followed by those coated with SALG and SA, showing an intermediate level. Uncoated fruits displayed the highest intensity of gray pulp symptoms (Fig. 4B). Aguirre-Joya et al. (2017) also reported higher percentage of gray pulp symptoms in uncoated than coated fruit when cold stored. Perhaps coatings could modify atmosphere that regulates ethylene production and reduce gray pulp development. However, no correlation was found between the ethylene values measured after the various storage periods plus 7 days of shelf life and the gray pulp intensity (Table 6). Nevertheless, these may be because ethylene

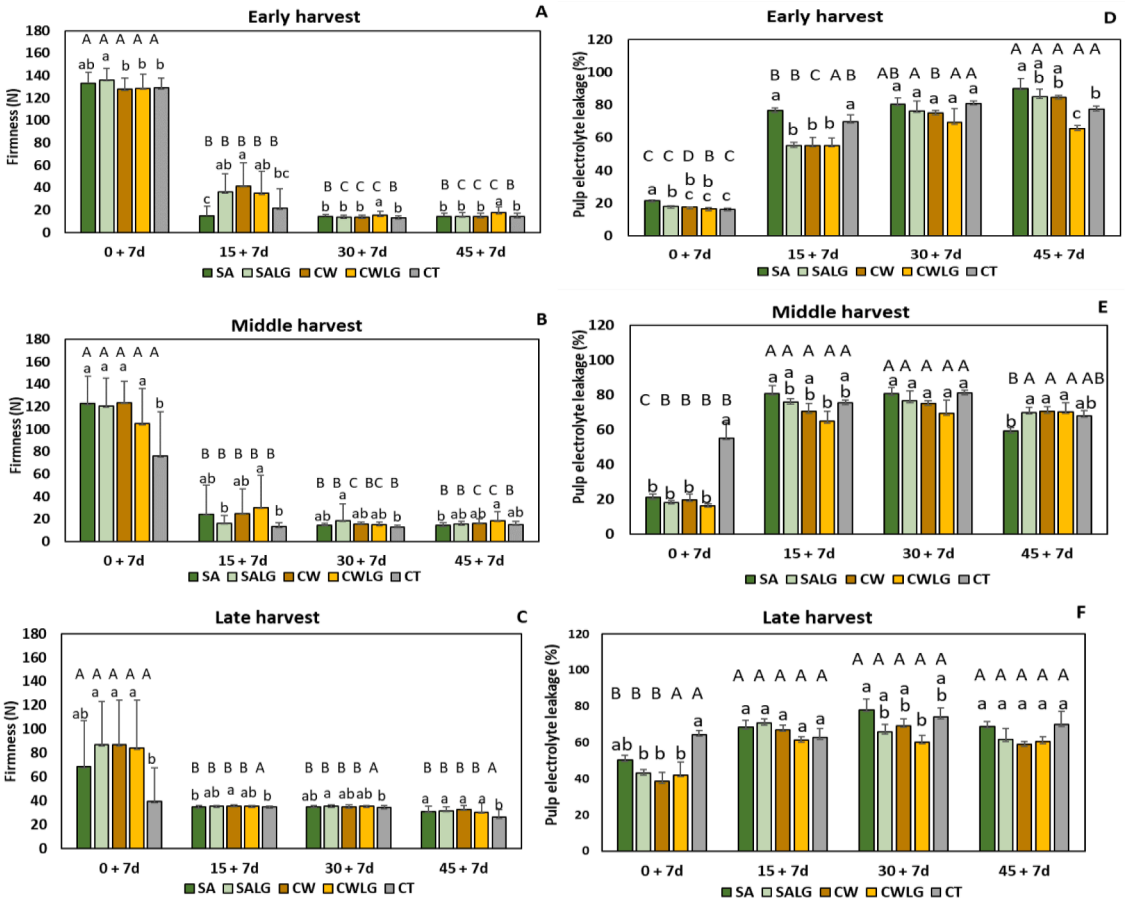

Fig. 2. Firmness (**A,B,C**) and pulp electrolyte leakage (**D,E,F**) of 'Hass' avocado with nanocoating's containing sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT), in fruit harvest in early (**A,D**), middle (**B,E**) and late (**C,F**) in the season, just after post-harvest treatment and after 15, 30 and 45 days of cold storage plus 7d shelf-life. Values are means ± standard error. For each storage period, bars with different lowercase show significant differences among treatments, while capital letters indicate significant differences among storage periods for the same treatment according to Duncan's multiple range test ($p < 0.05$).

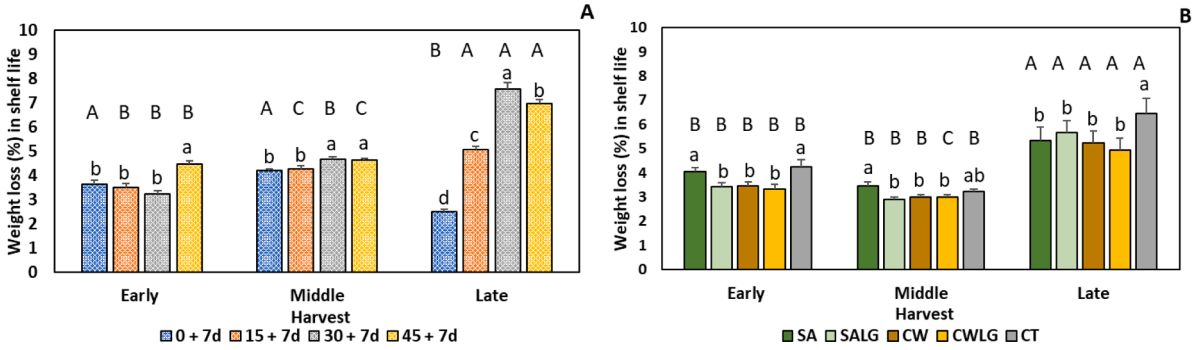

Fig. 3. Weight loss in shelf-life period of 'Hass' avocado. Effect of cold storage time (**B**); without cold storage (just after post-harvest treatment) plus 7 d shelf-life and after 15, 30 and 45 days of cold storage plus 7d shelf-life. Effect of postharvest treatments (**B**); nanocoating's containing sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT), in fruit harvest in early, middle, and late in the season. Values are means ± standard error. Bars with lowercase show significant differences among storage periods or treatments for each sampling date, while capital letters indicate significant differences between sampling periods according to Duncan's multiple range test ($p < 0.05$).

measurements were done just after 7 days shelf life, so the climacteric ethylene peak, could already have been attained (Hershkovitz et al., 2009; Farneti et al., 2015). The better effect of CW in reducing gray pulp in comparison to SA and control (Fig.4B) may be due to the lipidic coating composition which may provide better protection (Aloui et al., 2015; Miranda et al., 2020; Mpai and Sivakumar, 2020).

3.5.1. *α-Farnesene and conjugated trienols*

Physiological mesocarp discoloration (gray pulp) in avocado, like superficial scald in apple and pear have widely been associated to ethylene production (Hershkovitz et al., 2009; Guerra et al., 2012; Farneti et al., 2015). The most generally approved theory to explained scald development relates the appearance of the disorder to the

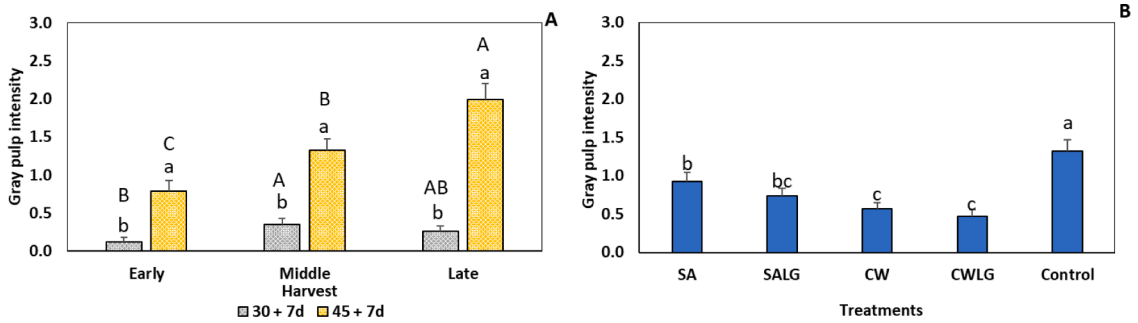

Fig. 4. Gray pulp intensity symptoms in 'Hass' avocado fruit harvested in early, middle, and late season **(A)** and with nanocoating's containing alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1 % (w/w) (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and control (fruit as it comes from the field) **(B).** Values are means ± standard error. Bars with different lowercase show significant differences between storage periods (A) or treatments (B), while capital letters indicate significant differences between sampling periods (A) according to Duncan's multiple range test ($p < 0.05$).

production of Farn and their oxidation into Ctrols (Farneti et al., 2015; Fonseca et al., 2020). However, to our knowledge, the possibility that the production of Farn and Ctrols could be associated with symptoms of gray pulp has never been explored. The results of the quantification of Farn ($p = 0.589$) and Ctrols ($p = 0.079$) in avocado pulp showed that the treatments had no effect on these parameters (Table 4). However, both parameters were affected by cold storage time ($p < 0.001$) and harvest date ($p < 0.001$) and had significant 3-way interaction between these two factors and treatment ($p = 0.031$ and $p = 0.041$, respectively for Farn and Ctrols) (Table 4). Focusing on the main effects it was found that both parameters increased from early to late harvest season, and with the increase in cold storage time. In fact, although there is no apparent effect of the treatments used in this work on Farn and Ctrols, it is visible that when they have higher values (mainly in the last harvest), the gray pulp development is higher (Table 4 and Fig. 4), suggesting an evolvement of those compounds on gray pulp development in avocado, which needs to be further investigated. Chanasut et al. (2018) reported a correlation between Farn and Ctrols with chilling injury in tangerine fruit. They studied early and late-season fruit and found higher chilling injury in the early-season harvest coincident with lower Farn than in the middle season. In our case, a similar pattern was observed, higher gray pulp with higher Farn, but in late-season. However, although there (Arnon et al., 2014; Glowacz et al., 2017) was also an increase in Ctrols through harvest season, Chanasut et al. (2018) found no differences in those compounds in both seasons.

Concerning the relation of Farn and Ctrols with ethylene production, the significantly higher content of Farn and Ctrols in late harvest and end of storage, coincides with higher significant ethylene production (Hershkovitz et al., 2009; Guerra et al., 2012; Farneti et al., 2015), although this effect was due to increased ethylene in control, while no differences among treatments were observed.

Table 4
The influence of harvest date (early, middle, and late), cold storage time (0 + 7d, 15+7d, 30+7d and 45+7d) and postharvest treatment [sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT)] on α-farnesene and conjugated trienols content in the pulp of 'Hass' avocado.

	α-Farnesene	Conjugated Trienols
Harvest date		
Early	124.92±11.96**c**	4.04**c**±0.42**c**
Middle	263.09±21.25**b**	5.67**b**±0.47**b**
Late	839.37±34.37**a**	16.76**a**±0.96**a**
Cold storage		
0 days	281.83±50.76**c**	5.92±1.08**c**
15 days	387.54±47.84**b**	8.19±0.81**b**
30 days	423.37±38.15**b**	7.7 ± 0.56**b**
45 days	543.75±52.41**a**	13.49±1.34**a**
Treatment		
SA	428.61±59.79**a**	7.87±1.10**a**
SALG	393.35±57.05**a**	8.07±1.17**a**
CW	410.09±53.36**a**	9.85±1.26**a**
CWLG	382.2 ± 54.36**a**	8.65±0.95**a**
CT	431.36±49.88**a**	9.69±1.39**a**
Factor		
Harvest (H)	**	**
Storage (S)	**	**
Treatment (T)	NS	NS
H *x* S	**	**
H *x* T	NS	*
S *x* T	NS	**
H *x* S *x* T	*	*

Values are means ± standard error. The values followed by the same letter, in the same column and factor (harvest date, cold storage and treatment) are not significantly different (Duncan's New Multiple Range Test, at $P = 0.05$). NS= non-significant;.
** Factor significant, $p < 0.01$;.
* Factor significant, $p < 0.05$.

3.6. Fatty acids

There were identified 9 main fatty acids in pulp avocado (Table 5), being the most abundant ones, oleic acid, palmitic acid, linoleic acid and palmitoleic acid as reported by other authors (Glowacz et al., 2017; Mpai and Sivakumar, 2020).

Harvest time and storage affected significantly all identified fatty acids as reported by other authors (Ozdemir and Topuz, 2004). The higher ratio of unsaturated/saturated fatty acids were found in the middle harvest, followed by latter and early harvest (Table 5). Cold storage time decreased capric acid (1) and oleic acid (5) and increased myristic acid (2), palmitic acid (3), palmitoleic acid (4), linolelaidic acid (6), linoleic acid (7), arachidic (8) and linolenic acid (9), the changes occurring mostly from 15 to 30 days. However, although reduced ratio of UFA/SFA is observed from 15 to 30 days, values did not show statistically significant changes (Table 5).

However, treatments had effect only in the fatty acids 4 (palmitoleic acid) (lower values in SA), 7 (linoleic acid) and 9 (α-linolenic acid) (higher values in control, for both). Nevertheless, the ratio UFA/SFA, showed no differences among treatments (Table 5). In fact, we found correlation only between the linolenic fatty acid and the gray mold symptoms (Table 6), with a significant increase from 15 to 30 days, coincident with the appearance of the first symptoms of gray pulp disorder. Glowacz et al. (2017) found reduced chilling injury in 'Hass' avocado by treatment with methyl jasmonate, coincident with increasing of the main unsaturated fatty acid (oleic acid) and the UFA/SFA present in the fruit. In our research, the edible coatings used appear to not influence the UFA/SFA, despite their influence on reducing gray pulp.

Table 5
The influence of harvest date (early, middle, and late), cold storage time (0 + 7d, 15+7d, 30+7d and 45+7d) and postharvest treatment [sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT)] on the oil content ratio (% on the total fatty acid – FAMEs content) of the main fatty acids (express in percentage) in the pulp of 'Hass' avocado.

	1	2	3	4	5	6	7	8	9	UFA/SFA
Harvest date										
Early	0.04 ± 0.00**a**	0.07 ± 0.00**a**	27.1 ± 0.17**a**	13 ± 0.95**a**	40.2 ± 0.43**c**	1.05 ± 0.06**b**	18.2 ± 0.19**a**	0.09 ± 0.00**a**	0.18**c** ± 0.00**c**	2.67 ± 0.02**c**
Middle	0.03 ± 0.00**b**	0.06 ± 0.00**b**	22.8 ± 0.27**c**	10.8 ± 0.09**b**	47.2 ± 0.36**b**	1.79 ± 0.05**a**	17 ± 0.15**b**	0.08 ± 0.00**b**	0.21**b** ± 0.00**b**	3.41 ± 0.09**a**
Late	0.01 ± 0.00**c**	0.05 ± 0.00**c**	23.9 ± 0.16**b**	10.5 ± 0.08**c**	50.4 ± 0.28**a**	0.63 ± 0.03**c**	14.2 ± 0.09**c**	0.08 ± 0.00**b**	0.22**a** ± 0.00**a**	3.18 ± 0.03**b**
Cold storage										
0 days	0.04 ± 0.00**a**	0.05 ± 0.00**d**	24.3 ± 0.32**b**	11.3 ± 0.20**bc**	47.3 ± 0.56**a**	1.14 ± 0.11**bc**	15.6 ± 0.21**c**	0.08 ± 0.00**b**	0.19 ± 0.00**b**	3.12 ± 0.06**a**
15 days	0.03 ± 0.00**b**	0.06 ± 0.00**c**	24.3 ± 0.43**b**	11.3 ± 0.20**c**	46.2 ± 0.80**b**	1.23 ± 0.09**a**	16.7 ± 0.32**ab**	0.09 ± 0.00**a**	0.19 ± 0.00**b**	3.18 ± 0.12**a**
30 days	0.02 ± 0.00**c**	0.06 ± 0.00**b**	25.2 ± 0.29**a**	11.7 ± 0.16**a**	44.7 ± 0.68**c**	1.06 ± 0.08**c**	17 ± 0.28**a**	0.08 ± 0.00**b**	0.21 ± 0.00**a**	2.97 ± 0.05**a**
45 days	0.02 ± 0.00**d**	0.06 ± 0.00**a**	24.6 ± 0.39**ab**	11.5 ± 0.23**ab**	45.6 ± 0.92**bc**	1.2 ± 0.08**ab**	16.6 ± 0.35**b**	0.09 ± 0.00**ab**	0.21 ± 0.00**a**	3.07 ± 0.06**a**
Treatment										
SA	0.03 ± 0.00**a**	0.06 ± 0.00**a**	24.4 ± 0.38**a**	11.3 ± 0.22**b**	46.7 ± 0.85**a**	1.15 ± 0.11**a**	16.2 ± 0.32**c**	0.09 ± 0.00**a**	0.2 ± 0.01**a**	3.11 ± 0.06**a**
SALG	0.03 ± 0.00**a**	0.06 ± 0.00**a**	24.8 ± 0.32**a**	11.6 ± 0.24**a**	45.7 ± 0.81**a**	1.12 ± 0.10**a**	16.4 ± 0.33**bc**	0.09 ± 0.00**a**	0.2 ± 0.01**a**	3.02 ± 0.05**a**
CW	0.03 ± 0.00**a**	0.06 ± 0.00**a**	25 ± 0.34**a**	11.6 ± 0.18**a**	45.6 ± 0.79**a**	1.13 ± 0.09**a**	16.3 ± 0.32**c**	0.09 ± 0.00**a**	0.2 ± 0.00**a**	2.99 ± 0.06**a**
CWLG	0.03 ± 0.00**a**	0.06 ± 0.00**a**	24.6 ± 0.37**a**	11.3 ± 0.24**ab**	45.9 ± 0.86**a**	1.18 ± 0.09**a**	16.7 ± 0.33**ab**	0.09 ± 0.00**a**	0.2 ± 0.00**a**	3.08 ± 0.06**a**
CT	0.03 ± 0.00**a**	0.06 ± 0.00**a**	24.2 ± 0.57**a**	11.6 ± 0.23**a**	45.7 ± 0.97**a**	1.21 ± 0.12**a**	16.9 ± 0.40**a**	0.09 ± 0.00**a**	0.21 ± 0.01**a**	3.22 ± 0.15**a**
Factor										
Harvest (H)	**	**	**	**	**	**	**	**	**	**
Storage (S)	**	**	*	**	**	**	**	*	**	NS
Treatment (T)	NS	NS	NS	*	NS	NS	**	NS	**	NS
H *x* S	**	**	*	**	**	**	**	**	**	NS
H *x* T	**	NS	NS	*	NS	**	*	NS	NS	NS
S *x* T	NS	NS	NS	*	NS	**	*	NS	NS	NS
H *x* S *x* T	**	**	NS	*	NS	**	*	**	NS	NS

1= Capric acid (C10:0); 2= Myristic acid (14:0); 3= Palmitic acid (C16:0); 4= Palmitoleic acid (C16:1n7); 5= Oleic acid (C18:1n9c); 6= Linolelaidic acid (C18:2n6t); 7= Linoleic acid (C18:2n:6c); 8= Arachidic acid (C20:0); 9= Linolenic acid (C18:3n3); UFA/SFA= unsaturated/saturated fatty acids. Values are means ± standard error. The values followed by the same letter, in the same column and factor (harvest date, cold storage and treatment) are not significantly different (Duncan's New Multiple Range Test, at $P = 0.05$). NS= non-significant;.
** Factor significant, $p < 0.01$;.
* Factor significant, $p < 0.05$.

Table 6
Pearson's correlations between firmness, pulp electrolyte leakage, gray pulp, and ethylene production, α-farnesene, conjugated tocotrienols, hydrogen peroxide content (H_2O_2), malondialdehyde content (MDA), fatty acids in 'Hass' avocado pulp.

Pearson's Correlations

Parameter	Firm	Pulp EL	Gray pulp	Ethy	Farn	Ctrlos	H2O2	MDA	1	2	3	4	5	6	7	8	9	UFA
Firm	1	-0.96**	-0.34**	0.11	-0.23	-0.27*	-0.62**	-0.41**	0.49**	-0.19	-0.04	-0.03	0.17	-0.16	-0.30*	-0.14	-0.33*	0.04
Pulp EL	-0.96*	1	0.26*	-0.10	0.20	0.24	0.56**	0.32*	-0.44**	0.18	0.08	0.06	-0.18	0.10	0.29*	0.10	0.30*	-0.07
Gray pulp	-0.34*	0.26*	1	-0.24	0.38*	0.54**	0.21	0.63*	-0.22	-0.01	-0.15	-0.14	0.17	-0.04	-0.14	0.04	0.45**	0.15

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

3.6.1. *Hydrogen peroxide and malondialdehyde*

Hydrogen peroxide itself is a signaling molecule in plants and is involved in the regulation of various physiological processes. In the context of avocado postharvest, the presence of hydrogen peroxide may have both positive and negative implications (Vincent and Munné-Bosch, 2022; Uarrota et al., 2022). On the positive side, low levels of hydrogen peroxide can serve as a signaling molecule to trigger defense mechanisms against pathogens and contribute to the regulation of ripening processes. However, excessive production of hydrogen peroxide can lead to oxidative stress, causing damage to cellular

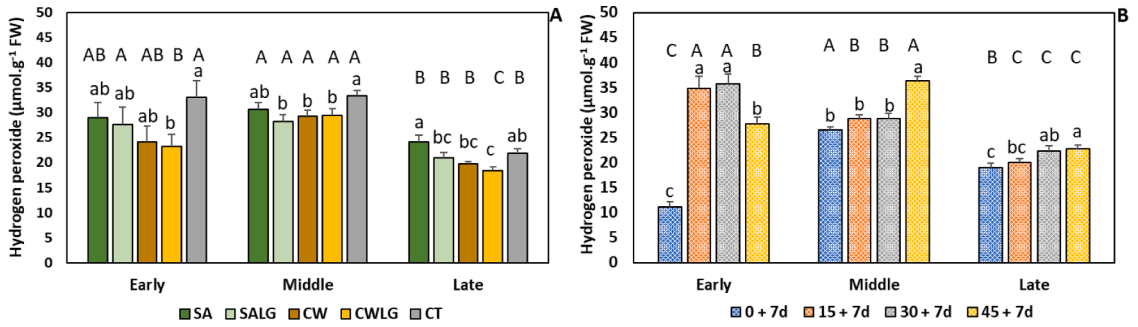

Fig. 5. Hydrogen peroxide content in 'Hass' avocado pulp. Effect of postharvest treatments (**A**); nanocoating's containing sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT), in fruit harvest in early, middle, and late in the season. Effect of cold storage time (**B**); without cold storage (just after post-harvest treatment) plus 7 d shelf-life and after 15, 30 and 45 days of cold storage plus 7d shelf-life. Values are means ± standard error. Bars with different lowercase show significant differences between storage periods (A) or treatments (B), while capital letters indicate significant differences between sampling periods (A) according to Duncan's multiple range test ($p < 0.05$).

components such as proteins, lipids, and DNA. This oxidative damage can result in accelerated senescence and the development of undesirable characteristics in the avocado, such as tissue browning.

The hydrogen peroxide in avocado was affected by harvest date ($p < 0.001$), cold storage time ($p < 0.001$) and treatment ($p < 0.001$), and there was a significant interaction between harvest date and cold storage time ($p < 0.001$). Control and SA-coated avocados showed the highest hydrogen peroxide content (Fig. 5A). Hydrogen peroxide levels were higher in the early and middle harvest than in the last harvest. There was an increase in values over storage time, but at early harvest the increase was much higher from 0 to 15 days storage, probably due to a greater sensitivity of early harvest fruit to cold storage (Fig. 5B). We found no correlation between hydrogen peroxide and gray pulp (Table 6).

The malondialdehyde content in fruit pulp was affected by harvest date ($p < 0.001$), cold storage time ($p < 0.001$) and treatment ($p < 0.001$), an interaction between treatment and cold storage time ($p < 0.001$) and an interaction between harvest date and cold storage time ($p < 0.001$).

MDA values increased in all treatments throughout the storage period mainly from 15 to 30 days, (Fig. 6A), coincident with the start of gray pulp development, showing a positive correlation with it (Table 6). Elevated levels of fruit lipid peroxidation leading to the buildup of MDA are frequently regarded as a sign of chilling injury (Wongsheree et al., 2009), which subsequently impacts fruit quality. The MDA content increased with cold storage time and from early harvest to late harvest in season (Fig. 6B). Also, Tesfay & Magwaza (2017) reported the same MDA content trend in avocado fruit during cold postharvest storage, followed by ripening under ambient conditions, indicating a tendency for higher increases in control fruits as compared to chitosan-coated ones.

3.7. Parameters correlation

The Pearson's correlation shows positive correlation of gray pulp with EL, farnesene, trienols, MDA and linoleic acid and negative with firmness (Table 6). In fact, electrolyte leakage is related to fatty acid changes and cell membrane disruption (Antunes and Sfakiotakis, 2008) which may be the one of the starters of the physiological disorder gray pulp.

It has been shown that farn and CTrols correlated to storage physiological disorders as superficial scald in pears (Guerra et al., 2012), which is confirmed in our work for the gray pulp disorder in avocado (Table 6). Changes in MDA have been also observed in cold stored avocado physiological disorders (Tesfay and Magwaza, 2017). The negative correlation with firmness indicates that, as more ripen is the fruit, more susceptible to gray pulp disorder, what is confirmed in our work, due to the higher expression of the disorder in late harvest avocado fruit.

3.8. Multivariate analysis

By the application of PCA to the analytical variables (all the parameters measured in avocado uncoated and coated with SA, SALG, CW

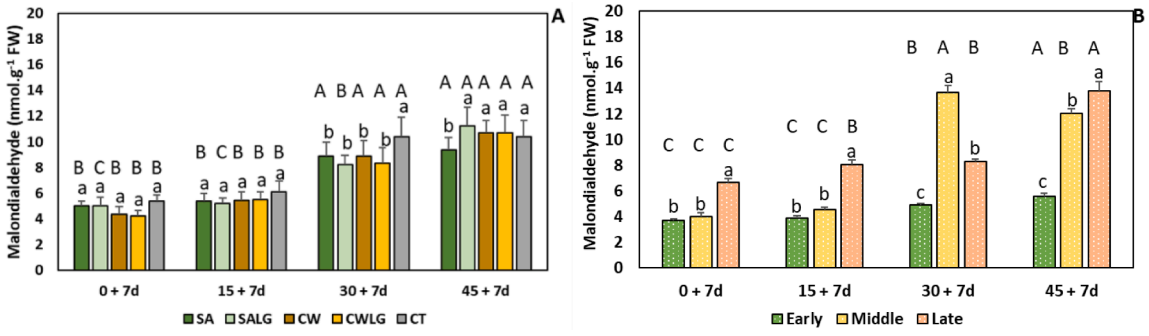

Fig. 6. Malondialdehyde content in 'Hass' avocado pulp. Effect of postharvest treatments (**A**); nanocoating's containing sodium alginate 2% (w/w) (SA), sodium alginate 2% (w/w) + lemongrass essential oil 1.25% (w/ w) (SALG), carnauba wax 1% (CW), carnauba wax 1% (w/w) + lemongrass essential oil 1.25% (w/w) (CWLG) and uncoated fruit (CT), and cold storage time [0 days (at harvest), 15 days, 30 days and 45 days + shelf-life]. Effect of harvest date [early, middle, and late] and cold storage time (**B**); [without cold storage (just after post-harvest treatment) plus 7 d shelf-life and after 15, 30 and 45 days of cold storage plus 7d shelf-life]. Values are means ± standard error. For each storage period, bars with different lowercase show significant differences among treatments (A) or harvest dates (B), while capital letters indicate significant differences among storage periods for the same treatment or harvest date according to Duncan's multiple range test ($p < 0.05$).

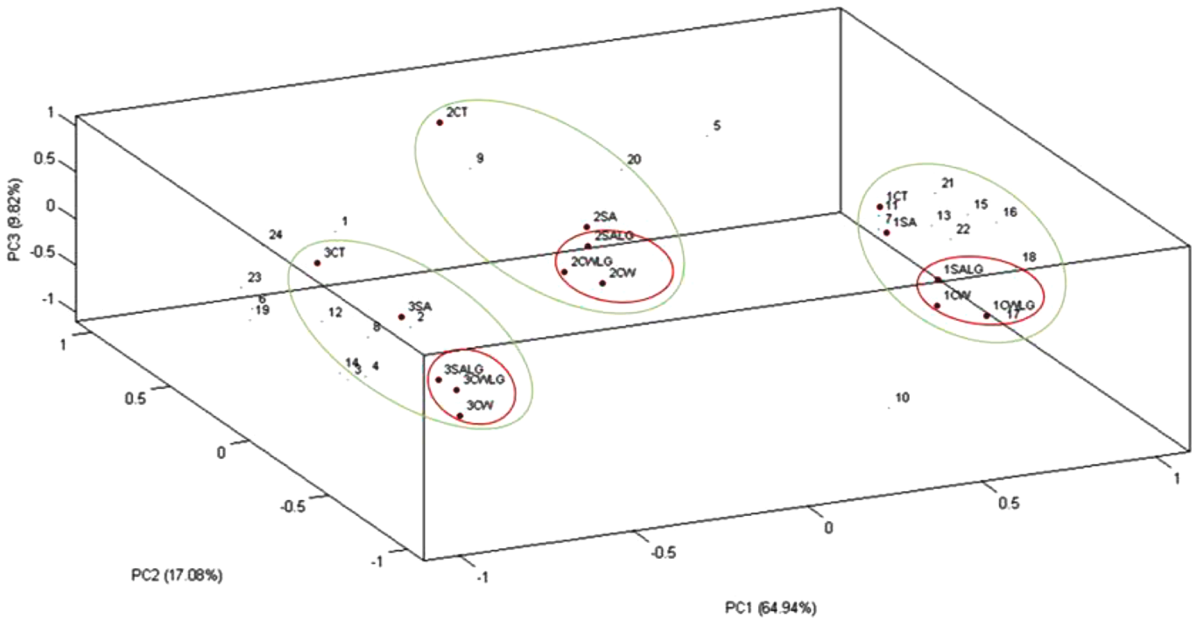

Fig. 7. Score and loading plots for PC1, PC2, PC3. 1= gray pulp; 2= ethylene; 3=α-farnesene; 4=conjugated trienols; 5= hydrogen peroxide; 6= malondialdehyde content; 7=hue pulp; 8 = *a**pulp; 9= electrolyte leakage; 10= firmness; 11=skin luminosity; 12=skin a* value; 13= skin hue; 14= skin luminosity; 15=Capric acid (C10:0); 16= Myristic acid (14:0); 17= Palmitic acid (C16:0); 18= Palmitoleic acid (C16:1n7); 19= Oleic acid (C18:1n9c); 20= Linolelaidic acid (C18:2n6t); 21= Linoleic acid (C18:2n:6c); 22= Arachidic acid (C20:0); 23= Linolenic acid (C18:3n3); 24= unsaturated fatty acid. The green lines encircle all treatments for each of the three harvests and the red lines enclose the three treatments with the closest projections.

and CWLG, in three harvest date and cold stored), three principal components (PCs) were extracted, explaining 91.64 % of the total variance. PC1 explains 64.94 %, PC2 17.08 % and PC3 9.82 % of the total variance (Fig. 7). The projections of the parameters measured, as well as position treatments at the 3 harvest dates along the three PCs are reported in Fig. 8. The PCs score plot showed a clear separation of the 3 harvest dates, with 3 groups of treatments with separated projections (the treatment acronym is preceded by 1, 2 and 3 respectively for early, middle, and late harvest). In all harvests, CW, CWLG and SALG are always together and separated from SA and mainly from control (Fig. 7). This enhances the benefit of CW as base coating, and the LG essential oil as additive. In fact, LG has proved to preserve quality mainly due to its antimicrobial and antioxidant capacity (Gago et al., 2020; Mpai and Sivakumar, 2020). The SA composed mainly by carbohydrates has a hydrophilic characteristic, leading to higher weight loss and less quality, while CW with its lipidic composition gives a better protection to avocado fruit (Oliveira Filho et al., 2022).

4. Conclusion

Avocado fruit harvested at different dates has influence in the post-harvest ripening and development of physiological disorders. For the 3 harvest times, the main changes in ripening parameters were from middle to late harvest, being the maximum cold storage extension of 45 days.

The coatings studied were effective in reducing ripening and ethylene production, as well as gray pulp development, being this effect higher in the first harvest. In the middle and late harvests, the CWLG or CW showed better results. The gray pulp physiological disorder was higher as the harvest time was delayed, being the highest in the late harvest. This disorder was shown only after 30 days of cold storage plus 7 days shelf life, coincident with the increase in MDA and linolenic acid. Nanocoatings were effective in reducing gray pulp physiological disorder, especially the CW or CWLG, which need further studies for the best concentrations to obtain the best results.

CRediT authorship contribution statement

Custódia Gago: Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Adriana Guerreiro:** Visualization, Methodology, Formal analysis. **Mariana Souza:** Formal analysis. **Nuno Martins:** Methodology, Formal analysis. **Daniela Fonseca:** Methodology, Formal analysis. **Maria João Cabrita:** Validation, Methodology, Investigation, Formal analysis. **Maria da Graça Miguel:** Visualization, Validation, Methodology, Investigation. **Maria Dulce Antunes:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Aguirre-Joya, J.A., Ventura-Sobrevilla, J., Martínez-Vazquez, G., Ruelas-Chacón, X., Rojas, R., Rodríguez-Herrera, R., Aguilar, C.N., 2017. Effects of a natural bioactive coating on the quality and shelf life prolongation at different storage conditions of

avocado (Persea americana Mill.) cv. Hass. Food Packag. Shelf Life 14, 102–107. https://doi.org/10.1016/j.fpsl.2017.09.003.
Aloui, H., Licciardello, F., Khwaldia, K., Hamdi, M., Restuccia, C., 2015. Physical properties and antifungal activity of bioactive films containing Wickerhamomyces anomalus killer yeast and their application for preservation of oranges and control of postharvest green mold caused by Penicillium digitatum. Int. J. Food Microbiol. 200, 22–30. https://doi.org/10.1016/j.ijfoodmicro.2015.01.015.
Antunes, M.D., Gago, C.M., Cavaco, A.M., Miguel, M.G., 2012. Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. Recent Patents Food, Nutr. Agric. 4. https://doi.org/10.2174/2212798411204020114.
Antunes, M.D.C., Sfakiotakis, E.M., 2008. Changes in fatty acid composition and electrolyte leakage of 'Hayward' kiwifruit during storage at different temperatures. Food Chem 110, 891–896. https://doi.org/10.1016/J.FOODCHEM.2008.02.089.
Arancibia-Guerra, C., Núñez-Lillo, G., Cáceres-Mella, A., Carrera, E., Meneses, C., Kuhn, N., Pedreschi, R., 2022. Color desynchronization with softening of 'Hass' avocado: Targeted pigment, hormone and gene expression analysis. Postharvest Biol. Technol. 194 https://doi.org/10.1016/j.postharvbio.2022.112067.
Arpaia, M.L., Collin, S., Sievert, J., Obenland, D., 2018. 'Hass' avocado quality as influenced by temperature and ethylene prior to and during final ripening. Postharvest Biol. Technol. 140, 76–84. https://doi.org/10.1016/j.postharvbio.2018.02.015.
Arnon, H., Zaitsev, Y., Porat, R., Poverenov, E., 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. Postharvest Biol. Technol. 87, 21–26. https://doi.org/10.1016/j.postharvbio.2013.08.007.
Chanasut, U., Rattanpanone, N., Boonyakiat, D., Kampoun, W., 2018. Chilling injury susceptibility of early-season "Sai Nam Peung" tangerine fruit and alteration of α-farnesene and conjugated trienols during low temperature storage. Chiang Mai J. Sci. 45, 147–153. https://epg.science.cmu.ac.th/ejournal/ContributedPaper.
Farneti, B., Busatto, N., Khomenko, I., Cappellin, L., Gutierrez, S., Spinelli, F., Velasco, R., Biasioli, F., Costa, G., Costa, F., 2015. Untargeted metabolomics investigation of volatile compounds involved in the development of apple superficial scald by PTR-ToF–MS. Metabolomics 11, 341–349. https://doi.org/10.1007/s11306-014-0696-0.
Fonseca, A.M.A., Dias, C., Amaro, A.L., Isidoro, N., Pintado, M., Silvestre, A.J.D., Rocha, S.M., 2020. The impact of plant-based coatings in "ROCHA" pear preservation during cold storage: A metabolomic approach. Foods 9. https://doi.org/10.3390/foods9091299.
Gago, C., Antão, R., Dores, C., Guerreiro, A., Miguel, M.G., Faleiro, M.L., Figueiredo, A. C., Antunes, M.D., 2020. The effect of nanocoatings enriched with essential oils on "Rocha" pear long storage. Foods 9. https://doi.org/10.3390/foods9020240.
Gago, C., Guerreiro, A., Cruz, S., Martins, N., Cabrita, M.J., Miguel, M.G., Faleiro, M.L., Antunes, M.D., 2022. 1-Methylcyclopropene and lemongrass essential oil nanocoatings effect on the preservation of cold stored ' Rocha ' pear. Postharvest Biol. Technol. 192, 1–10. https://doi.org/10.1016/j.postharvbio.2022.111992. Received.
Gago, C., Guerreiro, A., Miguel, M.G., Faleiro, M.L., Antunes, M.D., 2021. Stored 'Galia' melon quality affected by edible nanocoatings enriched with essential oils. Acta Hortic 1327, 583–590. https://doi.org/10.17660/ActaHortic.2021.1327.77.
Gago, C.M.L., Artiga-Artigas, M., Antunes, M.D.C., Faleiro, M.L., Miguel, M.G., Martín-Belloso, O., 2019. Effectiveness of nanoemulsions of clove and lemongrass essential oils and their major components against Escherichia coli and Botrytis cinerea. J. Food Sci. Technol. https://doi.org/10.1007/s13197-019-03762-1.
Gago, C.M.L., Guerreiro, A.C., Miguel, G., Panagopoulos, T., da Silva, M.M., Antunes, M. D.C., 2016. Effect of Calcium chloride and 1-MCP (Smartfresh™) postharvest treatment on 'Golden Delicious' apple cold storage physiological disorders. Sci. Hortic. (Amsterdam). 211 https://doi.org/10.1016/j.scienta.2016.09.017.
Gago, C.M.L., Miguel, M.G., Cavaco, A.M., Almeida, D.P.F., Antunes, M.D.C., 2015. Combined effect of temperature and controlled atmosphere on storage and shelf-life of "Rocha" pear treated with 1-methylcyclopropene. Food Sci. Technol. Int. 21 https://doi.org/10.1177/1082013213511808.
Germano, T.A., Aguiar, R.P., Bastos, M.S.R., Moreira, R.A., Ayala-Zavala, J.F., de Miranda, M.R.A., 2019. Galactomannan-carnauba wax coating improves the antioxidant status and reduces chilling injury of 'Paluma' guava. Postharvest Biol. Technol. 149, 9–17. https://doi.org/10.1016/j.postharvbio.2018.11.013.
Glowacz, M., Bill, M., Tinyane, P.P., Sivakumar, D., 2017. Maintaining postharvest quality of cold stored 'Hass' avocados by altering the fatty acids content and composition with the use of natural volatile compounds – methyl jasmonate and methyl salicylate. J. Sci. Food Agric. 97, 5186–5193. https://doi.org/10.1002/jsfa.8400.
Guerra, R., Gardé, I.V., Antunes, M.D., da Silva, J.M., Antunes, R., Cavaco, A.M., 2012. A possibility for non-invasive diagnosis of superficial scald in 'Rocha' pear based on chlorophyll a fluorescence, colorimetry, and the relation between α-farnesene and conjugated trienols. Sci. Hortic. (Amsterdam). 134, 127–138. https://doi.org/10.1016/J.SCIENTA.2011.11.017.
Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., Antunes, M.D.C., 2015. The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage. Postharvest Biol. Technol. 100, 226–233. https://doi.org/10.1016/J.POSTHARVBIO.2014.09.002.
Hashemi, S.M.B., Mousavi Khaneghah, A., 2017. Characterization of novel basil-seed gum active edible films and coatings containing oregano essential oil. Prog. Org. Coatings 110, 35–41. https://doi.org/10.1016/j.porgcoat.2017.04.041.
Hershkovitz, V., Friedman, H., Goldschmidt, E.E., Pesis, E., 2009. The role of the embryo and ethylene in avocado fruit mesocarp discoloration. J. Exp. Bot. 60, 791–799. https://doi.org/10.1093/jxb/ern328.
Iñiguez-Moreno, M., Ragazzo-Sánchez, J.A., César Barros-Castillo, J., Solís-Pacheco, R., Calderón-Santoyo, M., 2021. Characterization of sodium alginate coatings with Meyerozyma caribbica and impact on quality properties of avocado fruit. LWT - Food Sci. Technol. 152, 112346 https://doi.org/10.1016/j.lwt.2021.112346.
Jafarzadeh, S., Mohammadi Nafchi, A., Salehabadi, A., Oladzad-abbasabadi, N., Jafari, S. M., 2021. Application of bio-nanocomposite films and edible coatings for extending the shelf life of fresh fruits and vegetables. Adv. Colloid Interface Sci. 291, 102405 https://doi.org/10.1016/J.CIS.2021.102405.
Jo, W.S., Song, H.Y., Song, N.B., Lee, J.H., Min, S.C., Song, K., 2014. Quality and microbial safety of "Fuji" apples coated with carnauba-shellac wax containing lemongrass oil. LWT - Food Sci. Technol. 55, 490–497. https://doi.org/10.1016/j.lwt.2013.10.034.
Kubo, Y., Hiwasa, K., Owino, W.O., Nakano, R., Inaba, A., 2003. Influence of Time and Concentration of 1-MCP Application on the Shelf Life of Pear `La France' Fruit. HortScience 38, 1414–1416. https://doi.org/10.21273/HORTSCI.38.7.1414.
Kumar, R., Ghoshal, G., Goyal, M., 2019. Synthesis and functional properties of gelatin/CA–starch composite film: excellent food packaging material. J. Food Sci. Technol. 56, 1954–1965. https://doi.org/10.1007/s13197-019-03662-4.
Li, D., Zhang, X., Li, L., Aghdam, M.S., Wei, X., Liu, J., Xu, Y., Luo, Z., 2019. Elevated CO 2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. Food Chem 285, 163–170. https://doi.org/10.1016/j.foodchem.2019.01.150.
Maftoonazad, N., Ramaswamy, H.S., 2008. Effect of pectin-based coating on kinetics of quality change associated with stored avocados.
McClements, D.J., Henson, L., Popplewell, L.M., Decker, E.A., Choi, S.J., 2012. Inhibition of Ostwald ripening in model beverage emulsions by addition of poorly water soluble triglyceride oils. J. Food Sci. 77, C33–C38. https://doi.org/10.1111/j.1750-3841.2011.02484.x. PubMed.
McGuire, R.G., 1992. Reporting of Objective Color Measurements. HortScience 27, 1254–1255. https://doi.org/10.21273/hortsci.27.12.1254.
Medina-Carrillo, R.E., Salazar-García, S., Bonilla-Cárdenas, J.A., Herrera-González, J.A., Ibarra-Estrada, M.E., Álvarez-Bravo, A., 2017. Secondary metabolites and lignin in 'hass' avocado fruit skin during fruit development in three producing regions. HortScience 52, 852–858. https://doi.org/10.21273/HORTSCI11882-17.
Mendieta, B., Olaeta, J.A., Pedreschi, R., Undurraga, P., 2016. Reduction of cold damage during cold storage of Hass avocado by a combined use of pre-conditioning and waxing. Sci. Hortic. (Amsterdam). 200, 119–124. https://doi.org/10.1016/J.SCIENTA.2016.01.012.
Miranda, M., Ribeiro, M.D.M.M., Spricigo, P.C., Pilon, L., Mitsuyuki, M.C., Correa, D.S., Ferreira, M.D., 2022. Carnauba wax nanoemulsion applied as an edible coating on fresh tomato for postharvest quality evaluation. Heliyon 8, e09803. https://doi.org/10.1016/j.heliyon.2022.e09803.
Mpai, S., Sivakumar, D., 2020. Influence of growing seasons on metabolic composition, and fruit quality of avocado cultivars at 'ready-to-eat stage.'. Sci. Hortic. (Amsterdam) 265, 109159. https://doi.org/10.1016/j.scienta.2019.109159.
Nazoori, F., Mollai, S., Sobhani, F., Mirdehghan, S.H., Sahhafi, S.R., 2023. Carboxymethyl cellulose and carnauba wax treatments kept the pomegranate fruit (Punica granatum L.) quality during cold storage via improving enzymatic defense system and bioactive compounds. Sci. Hortic. (Amsterdam). 309 https://doi.org/10.1016/j.scienta.2022.111645.
Ngoc, T.T.B., Thu, V.M., Hien, N.T.P., Tra, H.T.T., An, H.D., Hue, T.T., 2022. Effect of exogenous melatonin on antioxidant enzyme activities and membrane lipid peroxidation in avocado fruit during ripening. Vietnam J. Biotechnol. 20, 495–504.
Nunes, C.A., Freitas, M.P., Pinheiro, A.C.M., Bastos, S.C., 2012. Chemoface: a novel free user-friendly interface for chemometrics. J. Br. Chem. Soc. 23, 2003–2010.
Ohashi, T.L., Pilon, L., Spricigo, P.C., Miranda, M., Corrêa, D., Souza, M., Ferreira, M.D., 2015. Postharvest quality of 'golden' Papayas (Carica papaya L.) coated with carnauba wax nanoemulsions. No Title. Rev. Iberoam. Tecnol. Postcosecha 16, 199–209.
Olivares, D., Alvarez, E., Véliz, D., García-Rojas, M., Díaz, C., Defilippi, B.G., 2020. Effects of 1-Methylcyclopropene and Controlled Atmosphere on Ethylene Synthesis and Quality Attributes of Avocado cvs. Edranol and Fuerte. J. Food Qual. 2020 https://doi.org/10.1155/2020/5075218.
Oliveira Filho, J.G.de, Silva, G.da C., Oldoni, F.C.A., Miranda, M., Florencio, C., Oliveira, R.M.D.de, Gomes, M.de P., Ferreira, M.D., 2022. Edible Coating Based on Carnauba Wax Nanoemulsion and Cymbopogon martinii Essential Oil on. Papaya Postharvest Preservation. Coatings 12, 1700. https://doi.org/10.3390/coatings12111700.
Osuna-García, J.A., Doyon, G., Salazar-García, S., Goenaga, R., González-Durán, I.J.L., 2011. Relationship between skin color and some fruit quality characteristics of "Hass" avocado. J. Agric. Univ. Puerto Rico 95, 15–23.
Otoni, C.G., Avena-Bustillos, R.J., Azeredo, H.M.C., Lorevice, M.V., Moura, M.R., Mattoso, L.H.C., McHugh, T.H., 2017. Recent Advances on Edible Films Based on Fruits and Vegetables—A Review. Compr. Rev. Food Sci. Food Saf. 16, 1151–1169. https://doi.org/10.1111/1541-4337.12281.
Ozdemir, F., Topuz, A., 2004. Changes in dry matter, oil content and fatty acids composition of avocado during harvesting time and post-harvesting ripening period. Food Chem 86, 79–83. https://doi.org/10.1016/j.foodchem.2003.08.012.
Pinto, H., Rueda-Chacón, J., Arguello, H., 2019. Classification of Hass avocado (persea americana mill) in terms of its ripening via hyperspectral images. TecnoLógicas 22, 111–130.
Ramírez-Gil, J.G., López, J.H., Henao-Rojas, J.C., 2020. Causes of hass avocado fruit rejection in preharvest, harvest, and packinghouse: Economic losses and associated variables. Agronomy 10. https://doi.org/10.3390/agronomy10010008.
Rocha, F.A.M., 2018. Divulgação Internacional do Potencial Vínico Português. Universidade de Évora 72. Pags.
Salvia-trujillo, L., Rojas-graü, M.A., Soliva-fortuny, R., Martín-belloso, O., 2015. Use of antimicrobial nanoemulsions as edible coatings: Impact on safety and quality

attributes of fresh-cut Fuji apples. Postharvest Biol. Technol. 105, 8–16. https://doi.org/10.1016/j.postharvbio.2015.03.009.

Shezi, S., Magwaza, L.S., Tesfay, S.Z., Mditshwa, A., 2020. Biochemical changes in response to canopy position of avocado fruit (cv. 'Carmen' and 'Hass') during growth and development and relationship with maturity. Sci. Hortic. (Amsterdam). https://doi.org/10.1016/j.scienta.2020.109227.

Sierra, N.M., Londoño, A., Gómez, J.M., Herrera, A.O., Castellanos, D.A., 2019. Evaluation and modeling of changes in shelf life, firmness and color of 'Hass' avocado depending on storage temperature. Food Sci. Technol. Int. 25, 370–384. https://doi.org/10.1177/1082013219826825.

Tesfay, S.Z., Magwaza, L.S., 2017. Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending postharvest life of avocado (Persea americana Mill.) fruit. Food Packag. Shelf Life 11, 40–48. https://doi.org/10.1016/j.fpsl.2016.12.001.

Tesfay, S.Z., Magwaza, L.S., Mbili, N., Mditshwa, A., 2017. Carboxyl methylcellulose (CMC) containing moringa plant extracts as new postharvest organic edible coating for Avocado (Persea americana Mill.) fruit. Sci. Hortic. (Amsterdam). 226, 201–207. https://doi.org/10.1016/j.scienta.2017.08.047.

Uarrota, V.G., Hernández, I., Ponce, E., Bauer, C.M., Maraschin, M., Pedreschi, R., 2022. Metabolic profiling and biochemical analysis of stored Hass avocado fruit by GC–MS and UHPLC-UV-VIS revealed oxidative stress as the main driver of 'blackspot' physiological disorder. Int. J. Food Sci. Technol. 57, 7896–7916. https://doi.org/10.1111/ijfs.16145.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective role of exogenous polyamines. Plant Sci 151, 59–66. https://doi.org/10.1016/S0168-9452(99)00197-1.

Vincent, C., Munné-Bosch, S., 2022. Quality determination of avocado fruit immersed in a pyridoxal 5′-phosphate solution. J. Food Compos. Anal. 110, 104526.

Wongsheree, T., Ketsa, S., Doorn, W.G.Van, 2009. The relationship between chilling injury and membrane damage in lemon basil (Ocimum × citriodourum) leaves. Postharvest Biol. Technol. 51, 91–96. https://doi.org/10.1016/j.postharvbio.2008.05.015.

Woolf, A.B., Cox, K.A., White, A., Ferguson, I.B., 2003. Low temperature conditioning treatments reduce external chilling injury of "Hass" avocados. Postharvest Biol. Technol. 28, 113–122. https://doi.org/10.1016/S0925-5214(02)00178-3.

Zauberman, G., Fuchs, Y., Akerman, M., 1985. Peroxidase activity in avocado fruit stored at chilling temperatures. Sci. Hortic. (Amsterdam). 26, 261–265. https://doi.org/10.1016/0304-4238(85)90113-X.