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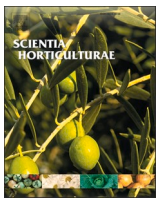

Application of polysaccharide-based edible coatings to improve the quality of zucchini fruit during postharvest cold storage

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ABSTRACT

The use of edible coatings has surged as a response to the ever-increasing demand for ecologically-friendly methods for maintaining fruit quality during storage. This study analyses the application of different polysaccharide-based coatings, carboxymethylcellulose, chitosan, dextrin and starch, and the use of a plasticizer agent, glycerol, with dextrin shown to be the most effective in maintaining the postharvest quality of zucchini fruit during cold storage. Subsequently, to improve these results, the nutraceutical additives oleuropein and olive oil, were tested in combination with dextrin. Results showed that dextrin coatings reduced weight loss, chilling injury, and oxidative stress in zucchini fruit at low temperature, maintaining fruit quality. The natural additives obtained from the olive tree showed a higher induction of antioxidant enzymes as well as a greater accumulation of ascorbate and total phenolics, with the dextrin coating with olive oil being even more effective in maintaining the chilling injury low until the end of storage, associated to phenolic metabolism. This type of preservation could be implemented for extending postharvest life and enhancing the overall quality of zucchini fruit.

1. Introduction

Zucchini is a subtropical horticultural crop whose immature fruit experiences chilling injury (CI) when stored below 10 °C (Carvajal et al., 2011). However, low temperature is employed to preserve the fruit during transport and commercialization to avoid the rapid deterioration that takes place after harvest. CI in fruit tissues causes changes in membrane lipids and cell wall structure, inducing softening, and the development of pitting in the fruit surface. In the region of pitting, the cells collapse and appear damaged, and all cellular layers that conform the exocarp tissue are affected (Carvajal et al., 2015a).

Different approaches have been assayed to delay the occurrence of CI, including the application of different metabolites such as abscisic acid, putrescine, γ-aminobutyric acid, and nitric oxide (Carvajal et al., 2017a; Jiménez-Muñoz et al., 2021; Palma et al., 2015, 2019). All of these chemical treatments maintained the postharvest quality of zucchini fruit during cold storage; however, amongst them, individually shrink wrapping of zucchini fruit reduced CI and oxidative stress most effectively (Megías et al., 2016). The use of plastic covers as a method for food preservation is extended in Europe for commercialization of different immature fruit such as cucumber and zucchini, but its main disadvantage is the generation of non-biodegradable plastic residues (Chen et al., 2021). Therefore, additional environmentally-friendly methods, that are low cost and easy to incorporate to the production chain, have to be tested to maintain the postharvest quality of immature fruit such as zucchini, with the same effectiveness as shrink wrapping.

The application of edible coatings, an alternative postharvest technology, has been widely studied in recent years with the aim of reducing the damages suffered by the fruit throughout the cold storage period. Edible coatings can be made up of proteins, lipids, or polysaccharides, and their combinations with other metabolites such as essential oils or phenolic compounds. Polysaccharides are the most employed compounds for fruit coating, with these being usually starch, chitosan, cellulose, pectin, and their derivatives, such as pullulan, carboxymethylcellulose, or alginates (Duong et al., 2022; Etemadipoor et al., 2020; Hassan et al., 2018; Shiekh et al., 2022). They act as a selective barrier against gases and solutes, preserving postharvest fruit quality, they can be safely ingested by consumers, and on some occasions, they also improve the organoleptic and nutritional quality of the commodities (Baldwin et al., 1995; Thakur et al., 2019).

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Starch is a polysaccharide composed of amylose and amylopectin, and it has been used to produce edible coatings due to its low cost and biodegradability (Gutiérrez et al., 2015; Schwach and Avérous, 2004). Starch-based coatings improve fruit quality during cold storage (García et al., 1998; Thakur et al., 2019). Carboxymethylcellulose is a cellulose derivative, and has been widely used because of its non-toxicity, biodegradability, and good film-forming ability (Yan et al., 2019). The application of carboxymethylcellulose-based coating has been proved to be an efficient treatment to preserve the postharvest quality of fruits such as pear (Hussain et al., 2010), mandarin (Ali et al., 2021) or pomegranate under cold stress (Nazoori et al., 2023).

Chitosan is a linear polysaccharide derived from chitin by deacetylation. Chitosan coatings are used to prolong the shelf life of fruit reducing softening and chilling injury (Mahmoudi et al., 2022; Zhang et al., 2015, 2022) and as an antimicrobial and antifungal compound (Aider, 2010; Lu et al., 2014).

Dextrin is a natural polysaccharide that is usually obtained from potato starch by physical or chemical breakdown (Andersson et al., 2002). The use of dextrin is much extended as a food additive due to its low cost. However, very few studies have analysed its potential also as a fruit coating (Amariz et al., 2010; Ribeiro et al., 2009).

To enhance the functionality of edible coatings, different additives such as antioxidants, nutraceuticals, antimicrobial, or plasticizing agents can be incorporated into the matrix (Jiang et al., 2012; Mannozzi et al., 2018; Özdemir and Gökmen, 2017). Glycerol is a plasticizer agent that is widely used in the formulation of several types of coatings, given its ability to improve the mechanical properties of biopolymers. Its presence leads to changes in the polysaccharide films structure due to the formation of new bonds (Cerqueira et al., 2012). However, glycerol is very hydrophilic and hygroscopic, so it can affect the permeability of gases and water vapour (Sothornvit and Krochta, 2001).

One of the main limitations of polysaccharide-based coatings is that they constitute poor moisture barriers, whereas lipidic coatings provide an effective waterproof barrier due to their hydrophobic nature (Debeaufort and Voilley, 2009). Nevertheless, lipids have a low mechanical integrity. For this reason, composite coatings between polysaccharides and lipids are being tested in fruit. A good candidate for mixing with polysaccharides is olive oil, due to its biochemical attributes and its extensive cultivation in the Mediterranean area (Ramana Rao et al., 2016). Olive oil production also generates different by-products such as olive leaves, which could be utilizes as a source of bioactive compounds (Berbel and Posadillo, 2018). Amongst them, oleuropein is the major phenolic component in olive leaf extract (Jemai et al., 2008). Oleuropein has antioxidative, antimicrobial, antiviral, cardioprotective, antihypertensive, and anti-inflammatory properties (Şahin and Bilgin, 2018). The supplementation of these types of compounds to an edible coating could provide an added value to the product, and also improve the postharvest quality of fruit.

The aim of this work was, in first place, to evaluate the effect of different polysaccharide-based edible coatings on the maintenance of zucchini fruit quality during cold storage, and subsequently, to study of influence of two natural additives obtained from olive trees on postharvest and nutritional quality of fruit.

2. Material and methods

2.1. Fruit material, treatments, and storage conditions

Zucchini fruit (*C. pepo* L. morphotype *Zucchini*) of the commercial hybrid cultivar "Sinatra" (Clause–Tezier) were provided by Fruits & Vegetables La ÑECA S.A.T. Sinatra fruit is very sensitive to chilling storage, and the fruit of this cultivar are harvested with a size of about 20 cm length and 5 cm diameter, a weight of between 250 g and 300 g, and at immature stage. During the experimentation time the maturity stage of fruit does not change.

In this study, fruit healthy, same maturation time and similar size were separated into three replicates per treatment and storage period, each one consisting of 6 fruits. Subsequently, two experiments were carried out in this work, and in both experiments the exocarp was chosen to test the biochemical parameters because during cold storage this tissue has been described as being the main affected in zucchini fruit (Carvajal et al., 2015a).

Experiment 1

Dextrin, starch, chitosan and carboxymethylcellulose were used for the coating formulations. Treatments were applied by submerging the fruit for 5 min at 20 °C in distilled water as the control (C), 1% (w/v) dextrin (D), 1% (w/v) starch (S), 1% (w/v) chitosan (CH), and 1% (w/v) carboxymethylcellulose (CMC). These polysaccharide-based coatings were used with and without 1.5% (v/v) glycerol in order to determine the effect of this plasticizer agent. The concentration used for each polysaccharide was chosen according to preliminary experiments (data not shown), and the percentage of plasticizer was determined according to the bibliography (Lima et al., 2010; Thakur et al., 2019; Torabi et al., 2020). After treatment, fruit were dried at room temperature for 2 h before being transferred to a controlled-environment chamber at 4 °C and 85–90% relative humidity (RH) in darkness. Weight loss (WL) and chilling-injury (CI) index were determined after 3, 7, 10 and 14 days of cold storage. At 14 days, the exocarp of each fruit was removed, mixed according to replicate, frozen, ground in liquid nitrogen, and stored at −80 °C.

Experiment 2

In this experiment, the following coatings were applied in the same manner as in experiment 1: distilled water as the control (C), 1% (w/v) dextrin (D), 1% (w/v) dextrin plus 0.3% (w/v) oleuropein (DO) and 1% (w/v) dextrin plus 0.2% (v/v) extra-virgin olive oil (DOO). Oleuropein was purified from olive tree leaves, and extra-virgin olive oil was purchased commercially. Fruits were stored at 4 °C for 14 days. For each biological replicate, the exocarp was removed and stored at −80 °C for the biochemical determinations.

2.2. Preparation of films

A dextrin solution was prepared in water at 65 °C under magnetic stirring for 1h Potato starch was gelatinized in water at 90 °C under magnetic stirring for 2h Chitosan was dispersed in a solution of 0.7% (w/v) glacial acetic acid in water under magnetic stirring for 2 h, after which the pH of the solution was neutralized with 1 M NaOH (Yan et al., 2019). A solution of carboxymethylcellulose was prepared in water at 80 °C under magnetic stirring for 2 h (Arnon et al., 2015). Glycerol and oleuropein were dissolved under magnetic stirring at room temperature, whereas extra-virgin olive oil was emulsified using Ultraturrax T25 homogenizer at 13,500 rpm for 4 min in the corresponding polysaccharide coating solutions (Vargas et al., 2006).

2.3. Weight loss and chilling-injury index

The percentage of weight loss of each fruit was calculated using the following formula:% weight loss $= (W_i - W_f)/W_i \times 100$, being Wi the initial fruit weight and W_f the final fruit weight.

Chilling injury index (CI) was evaluated using a subjective scale of visual symptoms described by Carvajal et al. (2011). Each fruit was rated according to the following scale: 0, no pitting; 1, slight (10% or less of pitting in fruit surface); 2, medium (10–20% of pitting in fruit surface); and 3, severe pitting (>20% of pitting in fruit surface).

2.4. Respiration rate

To measure respiration rate, two fruits were sealed in a 4 L glass chamber for 4 h at room temperature. After this incubation period, the percentage of CO_2 was determined with a Dansensor CheckPoint 3. Five replicates from two fruits were analysed for each treatment, and then these fruits were discarded. CO_2 production was expressed as mg per Kg

of fresh weight per hour.

2.5. Firmness

Firmness was measured using a fruit hardness tester TR TURONI-Italy model 53,205 fitted with 8 mm diameter plunger and expressed in Newtons (N). Measurements regarding firmness were taken on the equatorial zone on each fruit at 90°

2.6. Lipid peroxidation

Lipid peroxidation was measured by TBARS assay following the method proposed by Carvajal et al. (2017a). 0.5 g of exocarp powder were extracted with 1 mL 20% trichloroacetic acid (TCA) (w/v) and 0.2 mL 4% butylated hydroxytoluene (BHT) (w/v). The extract was centrifuged during 15 min at 10,000 xg and 4 °C. The reaction mixture was composed by 0.25 mL of supernatant and 0.75 mL of 0.5% thiobarbituric acid (TBA) (w/v) and the samples were incubated during 30 min at 94 °C, then the reaction was stopped in ice for 10 min and the samples were centrifuged again at 10,000 xg during 10 min at 4 °C. The absorbance of the supernatant was measured at 532 nm, and at 600 nm to measure the unspecific aaence. Results were calculated according to a calibration curve and expressed as μg of MDA per Kg of fresh weight.

2.7. Hydrogen peroxide content

H_2O_2 content was analysed as in Alexieva et al. (2001). Exocarp powder was homogenized with 0.1% (w/v) trichloroacetic acid (1:4, w/v) and centrifuged during 15 min at 4 °C and 12,000 xg. Reaction mixture was composed by 0.25 mL of supernatant, 0.25 mL of 0.1 M potassium phosphate buffer pH = 7 and 1 mL of 1 M KI. Samples were incubated 1 h in darkness at room temperature, and their aaence were measured at 390 nm. Results were calculated according to a calibration curve and expressed as mg of H_2O_2 per Kg of fresh weight.

2.8. Ascorbate content

Ascorbate was extracted following the method described by Carvajal et al. (2015b). It was then quantified by HPLC in an Agilent 1260 Infinity system equipped with an Agilent ZORBAX Eclipse plus C18 column (150 mm × 4.6 mm id, 3.5 μm) (flow rate: 0.4 mL min^{-1}; isocratic conditions: 85% Milli-Q water pH 3 and 15% methanol, run time of 7.5 min; detection: 254 nm). Results were calculated according to a calibration curve and expressed as mg of ascorbate per Kg of fresh weight.

2.9. Total phenolic tests

Exocarp sample was extracted with methanol 80% as described by Castro-Cegri et al. (2023). Total phenolic compounds were measured using the Folin–Ciocalteu reagent method, according to Singleton and Rossi (1965). Reaction mixture was composed by 0.1 mL of extract and 0.25 ml of 20% (v/v) diluted Folin-Cicolteau reagent. After agitation and incubation during 5 min in darkness at room temperature, 0.25 ml of 20% (w/v) Na_2CO_3 and 0.9 ml of distillate water were added. Samples were then incubated 30 min in darkness and centrifuged during 5 min at room temperature and 5000 xg. Supernatants absorbance was measured at 760 nm. Results were calculated according to a calibration curve prepared with gallic acid and expressed as mg per Kg of fresh weight.

2.10. Enzymatic activities

Superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), and catalase (CAT; EC 1.11.1.6), were extracted and assayed according to Carvajal et al. (2015). SOD was assayed according to Beyer and Fridovich (1987), and the results were expressed as units per s and Kg of fresh weight. APX was measured according to Hossain and Asada (1984), and the results were expressed as mg ascorbate per s and Kg of fresh weight. CAT was assayed following the method described by Arbona et al. (2008) measuring hydrogen peroxide content and expressed as μg H_2O_2 per s and Kg of fresh weight.

Polyphenol oxidase (PPO; EC 1.14.18.1) and peroxidase (POD; EC 1.11.1.7) extraction were performed by homogenizing the exocarp powder in 0.1 M sodium phosphate buffer pH 6.5 (1:2, w/v). Homogenates were centrifuged for 20 min at 4 °C and 20,000 x g, and supernatant proteins were precipitated with ammonium sulphate at 100% saturation. Precipitated proteins were collected by centrifugation for 15 min at 4 °C and 15,000 x g, resuspended in 3 mL of 0.1 M sodium phosphate buffer pH 6.5, and dialyzed at 4 °C in the same buffer. The reaction mixture for PPO activity was composed by 0.1 M sodium phosphate buffer, pH 6, 20 mM pyrocatechol, and 0.1 mL of protein extract. The increase of absorbence at 410 nm was recorded for 1 min, and the activity was calculated according to a calibration curve prepared with catechol and expressed as μg per s and Kg of protein. The reaction mixture for POD activity was composed by 0.1 M sodium phosphate buffer pH 6.8, 72 mM guaiacol, 118 mM hydrogen peroxide, and 0.1 mL of protein extract. POD was assayed by measuring absorbance at 470 nm, and the results were expressed as mg of H_2O_2 per s and Kg of protein.

2.11. Protein determination

Total protein concentration was measured using the Bradford assay (1976).

2.12. Statistical analysis

The experiments were completely randomized. The statistical analysis was performed with an ANOVA using the SPSS 25.0 program (SPSS Inc.). Means were compared with Duncan's least significant differences test ($p < 0.05$).

3. Results and discussion

3.1. Selection of a polysaccharide-based coating to maintain quality of zucchini fruit

To maintain fruit quality after harvest, different polysaccharide-based coatings have been described for low temperature storage (Ali et al., 2011; Kumar et al., 2017; Nazoori et al., 2023; Valero et al., 2013). However, there is a very broad diversity of polysaccharide-based coatings, and differences amongst them have been reported for different fruits (Shiekh et al., 2022). In this work, the effect of several polysaccharides on postharvest quality maintenance of zucchini fruit during cold storage was studied. Zucchini fruit were treated with water (control), 1% (w/v) dextrin (D), 1% (w/v) starch (S), 1% (w/v) chitosan (CH) and 1% (w/v) carboxymethylcellulose (CMC), also with or without 1.5% (v/v) glycerol, and kept for 14 days at 4 °C.

The percentage of WL and CI index were evaluated throughout storage at 4 °C, and the results are shown in Table 1. At 14 days of cold storage, all plasticizer-free coatings reduced fruit WL with respect to control fruit, but the use of glycerol as a plasticizer did not have a significant effect on WL with respect to control fruit.

In zucchini squash, a reduction in WL has been reported when edible coatings such as gum arabic and fruwash were applied to fruit stored at 8 °C (Kannaujia et al., 2019). In our case, the use of glycerol as a plasticizer did not have a significant effect on WL, what could be explained since these plasticizers increase in fruit permeability, as they are incorporated to enhance the film's flexibility and resilience, which results in an increase in film porosity (Sothornvit and Krochta, 2005). Amongst all the polysaccharides used, dextrin was the only coating that decreased the CI after 14 days of storage at low temperature, whereas when glycerol was used as a plasticizer, the fruit experienced more

Table 1
Changes in percentage of weight loss and chilling-injury index of zucchini fruit control (C) and coated with 1% dextrin (D), 1% dextrin plus 1.5% glycerol ($D + G$), 1% starch (S), 1% starch plus 1.5% glycerol ($S + G$), 1% chitosan (CH), 1% chitosan plus 1.5% glycerol (CH + G), 1% carboxymethylcellulose (CMC) and 1% carboxymethylcellulose plus 1.5% glycerol (CMC+G) along the storage at 4 °C.

	Days at 4ºC	C	D	$D + G$	S	$S + G$	CH	CH + G	CMC	CMC + G	LSD ($p < 0.05$)
Weight loss (%)	3	05.67^{a}	4.68^{bcd}	05.06^{abc}	4.07^{d}	05.40^{ab}	4.55^{bcd}	05.08^{abc}	04.37^{cd}	04.46^{bcd}	0.66
	7	07.91^{a}	6.39^{bc}	07.17^{ab}	5.80^{c}	07.29^{ab}	6.06^{bc}	06.75^{abc}	06.50^{bc}	06.56^{bc}	0.86
	10	10.40^{a}	8.33^{b}	09.54^{ab}	8.10^{b}	09.16^{ab}	8.31^{b}	09.18^{ab}	08.66^{b}	09.36^{ab}	1.12
	14	11.75^{a}	9.45^{b}	10.77^{ab}	9.26^{b}	10.55^{ab}	9.50^{b}	10.51^{ab}	10.05^{b}	10.56^{ab}	1.17
Chilling-injury (0–3)	3	00.77^{a}	0.30^{ab}	00.25^{b}	0.31^{ab}	00.67^{ab}	0.33^{ab}	00.5^{ab}	00.75^{a}	00.42^{ab}	0.31
	7	01.41^{a}	0.50^{b}	00.67^{b}	1.15^{ab}	01.00^{ab}	0.75^{b}	00.58^{a}	01.50^{a}	01.42^{a}	0.44
	10	02.14^{ab}	1.00^{c}	01.25^{c}	1.69^{abc}	01.75^{abc}	1.50^{bc}	01.25^{c}	02.25^{ab}	02.33^{a}	0.53
	14	02.41^{a}	1.00^{b}	01.58^{ab}	2.00^{a}	02.00^{a}	2.17^{a}	01.82^{a}	02.33^{a}	02.58^{a}	0.55

Data presented are means of 18 fruits. Different letters for each storage time indicate significant differences between treatments according to Duncan's test ($p < 0.05$).

stress, as CI increased. In mango fruit, the use of different dextrin coatings reduced weight loss and preserved of fruit appearance, but only dextrin 2% with sunflower oil delayed the maturation, showing organic acids breakdown, and delay on the increase of pectic substances content (Ribeiro et al., 2009). Edible coatings based on polysaccharides can be used as a protective barrier to reduce respiration rate through fruit surfaces (Jafarzadeh et al., 2021), for zucchini fruit, chitosan and carboxymethylcellulose coatings were better at reducing the respiration rate with respect to control fruit after cold storage (Table 2). Although the respiration rate was measured after acclimatization of fruit to room temperature, the postharvest cold storage of zucchini for 14 days diminished the CO_2 production in all treatments with respect to freshly-harvested fruits. This response could be due to the occurrence of CI in fruit due to cold storage, in fact, CI has been reported to suppress respiration rate in mango fruit (Nair and Singh, 2009). However, Balandrán-Quintana et al. (2003) described an increase in respiration rate as an indicator of the next appearance of visible damage due to cold stress. Ours results showed that after 14 days of cold storage, the less injured fruit had a higher respiration rate. These fruits could be found close to a "burst" of respiration, as described in zucchini fruit prior to the appearance of visible damage on the surface of the fruit (Balandrán--Quintana et al., 2003).

Some of the main symptoms of CI in zucchini fruit are softening and lipid peroxidation (Carvajal et al., 2017b); fruit coated with dextrin and CMC showed higher firmness than the control after 14 days of cold storage (Table 2), but only the dextrin coating maintained the same firmness as freshly-harvested fruit, what is of great importance for the consumer's assessment of the fruit. In watermelon, a coating solution of alginate and cyclodextrin showed to maintain firmness in the fruit evaluated by a consumer acceptance text (Sipahi et al., 2013). The use of edible polysaccharide film has been reported to maintain firmness in cherries by reducing the activity of cell wall degrading enzymes (Afonso et al., 2023). For the dextrin coated fruit, lipid peroxidation was 30% lower than control fruit after storage at low temperature (Table 2). This parameter has been proven to be a good indicator of cold stress, and a positive correlation with chilling injury has been observed in zucchini fruit (Carvajal et al., 2015b).

With the results obtained, the dextrin-based coating was the film selected for further experiments, as it was able to reduce WL, CI and lipid peroxidation, and maintain firmness in fruit after 14 days at 4 °C. Furthermore, it was the most environmentally-friendly coating, as it requires less energy to prepare, because dextrin has greater solubility in water than other polysaccharide coatings and therefore less temperature is needed to carry out its dissolution (Islamipour et al., 2022).

However, these experiments are still preliminary and far from being compared to the use of plastic shrink wrapping, so further studies have to be conducted to improve the quality of zucchini fruit during cold stress. To achieve this, the coating with dextrin was supplemented with the additives olive oil and oleuropein, and an in-depth study of their effect on cold tolerance mechanisms was performed in experiment 2.

3.1.1. Effect of a dextrin and additive-based coating on cold tolerance of zucchini fruit

To enhance the properties of edible polysaccharide coatings, it is common to supplement them with essential oils, waxes, lipids, and purified antioxidant compounds (Guerreiro et al., 2015; Özdemir and Gökmen, 2017; Ribeiro et al., 2009). In fact, the application of chitosan plus pomelo extract significantly reduced browning, weight loss, and disease severity to extend shelf life of cold-stored lychee fruit (Yang et al., 2023). In this study, olive oil and oleuropein were added to dextrin, and their effect on maintaining quality was measured during low temperature storage. The treatments consisted in water (control), 1% (w/v) dextrin (D), 1% (w/v) dextrin plus 0.3% (w/v) oleuropein (DO) and 1% (w/v) dextrin plus 0.2% (v/v) extra-virgin olive oil (DOO). Polysaccharide-based films are not good barriers against water loss (Sothornvit and Krochta, 2005); therefore, these types of edible coatings could be improved for that purpose by the addition of hydrophobic materials such as olive oil (Guerreiro et al., 2015), to produce an emulsion-based edible film. Zucchini fruit embedded in dextrin supplemented with oleuropein, lost less weight than control fruits after 14 days of cold storage, and the same was observed for olive oil (Fig. 1), in this case probably due to the hydrophobicity of the oil and its ability to occupy amorphous regions of the polysaccharide coating (Shiekh et al., 2022). CI was high in control fruits, and a significant decrease in this disorder was detected in zucchini fruit covered with an edible coating (Fig. 1). The composite dextrin and olive oil showed only half of the CI observed with the other coatings, reducing CI by around 75% as compared to control fruit. In figs coated with composite chitosan and alginate emulsions of olive oil, a reduction of WL during cold storage was detected with respect to non-treated fruit, as well as a maintenance of firmness (Vieira et al., 2021). In tomato, a chitosan-olive oil emulsion also maintained the fruit firmness and delayed the ripening and

Table 2
Respiration rate (mg CO_2 h^{-1} Kg^{-1} FW), Firmness (N) and lipid peroxidation (μg MDA Kg^{-1} FW) in zucchini fruit freshly harvested or stored at 4ºC for 14 days with the following coatings: control (C) and coated with 1% dextrin (D), 1% dextrin plus 1.5% glycerol ($D + G$), 1% starch (S), 1% starch plus 1.5% glycerol ($S + G$), 1% chitosan (CH), 1% chitosan plus 1.5% glycerol (CH+G), 1% carboxymethylcellulose (CMC) and 1% carboxymethylcellulose plus 1.5% glycerol (CMC+G).

	At harvest	C	D	$D + G$	S	$S + G$	CH	CH + G	CMC	CMC + G	LSD ($p < 0.05$)
Respiration rate	82.73^{a}	68.82^{c}	75.03^{b}	70.92^{bc}	68.81^{c}	67.43^{c}	60.44^{d}	61.54^{d}	55.31^{e}	51.11^{f}	4.07
Firmness	09.35^{a}	04.67^{d}	08.68^{a}	06.53^{c}	05.80^{cd}	05.12^{d}	05.62^{cd}	07.62^{b}	06.32^{c}	05.65^{cd}	0.70
Lipid peroxidation	324^{c}	578^{ab}	408^{c}	653^{a}	403^{c}	632^{a}	434^{bc}	670^{a}	437^{bc}	580^{ab}	61.31

Data presented are means of triplicate samples of six fruit each. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

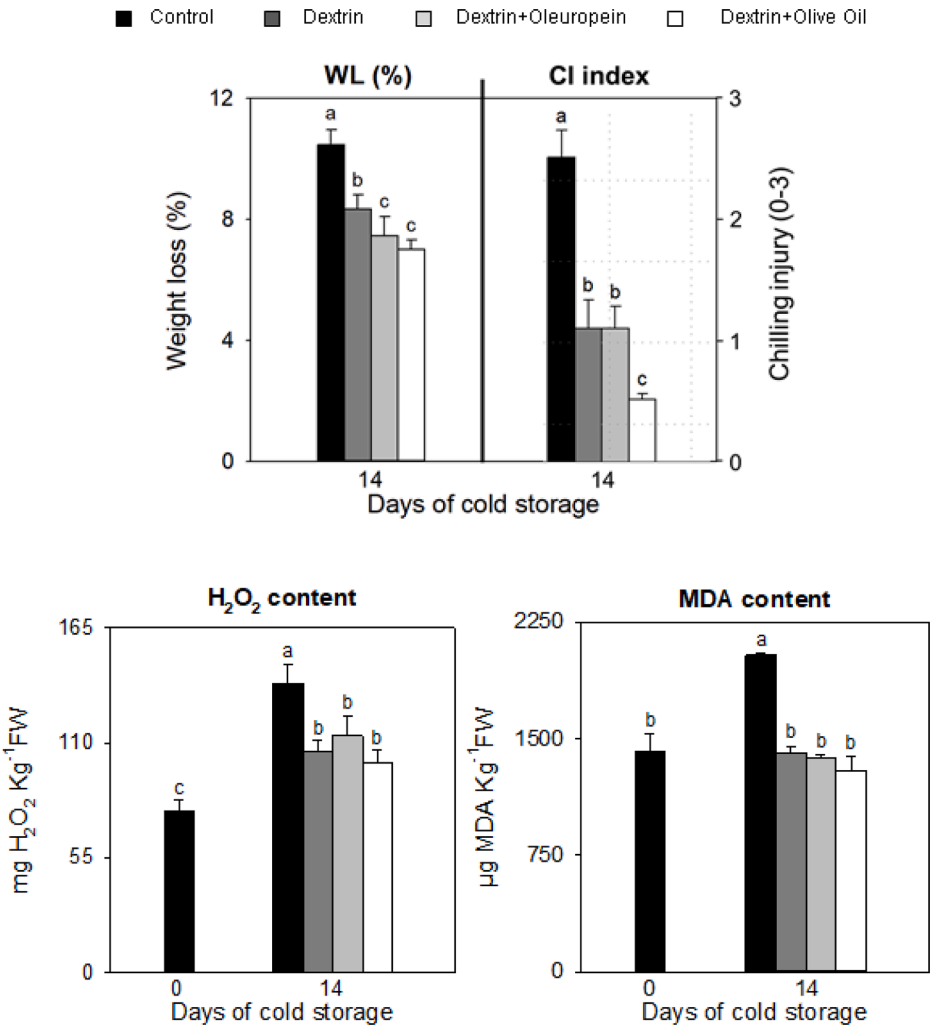

Fig. 1. Changes in percentage of weight loss (WL), chilling-injury index (CI), hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA) content of zucchini fruit control and coated with 1% dextrin (D), 1% dextrin plus oleuropein (DO) and 1% dextrin plus 0.2% olive oil stored during 14 days at 4 °C. Data presented in WL and CI are means of 18 fruit ± SE, and for H_2O_2 and MDA data are means ± SE of triplicate samples of six fruit each. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

(Dovale-Rosabala et al., 2015). Similar results were obtained in persimmon with gelatine and frog skin oil as a coating (Kingwascharapong et al., 2020), and in pear, pomegranate and star fruit chilling injuries were reduced with the use of lipid-based coatings (Adhikary et al., 2022; Barman et al., 2011; Zaki et al., 2012).

3.1.2. *Effect of a dextrin and additive-based coating on oxidative stress markers*

Cold storage is important for avoiding excessive fruit maturation during commercialization, but it also stresses tropical or subtropical fruit such as zucchini. This stress generates an increase in reactive oxygen species, such as H_2O_2. In Fig. 1 the levels of H_2O_2 at harvest and after 14 days of cold storage are shown. In all cases, there was an increase in this ROS by storage at 4 °C. However, the use of dextrin, with or without additives, was effective for the reduction of H_2O_2 content, with a 30% reduction detected with respect to control fruit, indicating a strong increase in the antioxidant mechanism induced by this coating. According to our results, Mahmoudi et al. (2022) observed that plum fruit treated with chitosan exhibited lower H_2O_2 content, resulting from the higher activity of antioxidant enzymes. The first event that causes chilling injury in cold-sensitive fruit is the loss of membrane integrity (Sevillano et al., 2009), usually evaluated as the level of lipid peroxidation, a parameter considered as a good indicator of chilling susceptibility in postharvest physiology. As shown in Fig. 1, all the dextrin coatings significantly decreased cold-induced membrane damage after 14 days at 4 °C, showing values equal to freshly-harvested fruit. Despite the fact that the phenolic compounds present in olive oil are potent antioxidants (Ricelli et al., 2020), and oleuropein is also a phenolic-derived compound with radical scavenging properties (Jemai et al., 2008), the use of both additives caused no significant differences with respect to a plain dextrin coating for lipid peroxidation, although the DOO treated fruit suffered of less damages since the CI in these fruits was the lowest. Fruit coating with chitosan and arginine nanoparticles delayed chilling injury by reducing oxidative stress, maintaining the membrane integrity, and controlling the accumulation of MDA (Mahmoudi et al., 2022). Dextrin coatings significantly improved the visual appearance and retarded the chilling injury of zucchini fruit by reducing the malondialdehyde and H_2O_2 content. Similar results have been described in rose apples with different alginate-based edible coatings (Duong et al., 2022).

3.1.3. *Effect of a dextrin and additive-based coating on antioxidant enzymes*

Cold storage increases the ROS levels in chilling-sensitive fruits, and the capacity of the tissue to remove ROS determines the adaptation of the fruit to stress conditions. Postharvest treatments that improve fruit performance at low temperature increase ROS-scavenging mechanisms (Carvajal et al., 2015b; Palma et al., 2016). The protection of fruit cells from chilling injury is directly related to the activity of antioxidant enzymes such as SOD, APX or CAT, which are key enzymes for the defence against ROS in the resistance of fruit to chilling (Jiménez-Muñoz et al., 2021; Sharma et al., 2020; Yao et al., 2018), and additives such as fatty acids can enhance this response in fruit (He et al., 2021).

SOD catalyses the disruption of the superoxide anion into hydrogen

peroxide, of great importance for maintaining a low cellular damage (Apel and Hirt, 2004). In DO and DOO-coated fruit, SOD activity was about 20% higher than the control after 14 days of cold storage (Fig. 2), what could help in maintaining a lower chilling injury index in these fruits. APX and CAT cooperate for protection against the H_2O_2 generated during low-temperature storage (Mizuno et al., 1998). In the case of APX, several coatings have been proven to increase its activity, helping to diminish the H_2O_2 concentration (Hu et al., 2022; Saleem et al., 2020). D- and DO-treated zucchini fruit had a higher activity of this antioxidant enzyme than control fruit, with the highest found in the fruit supplemented with oleuropein (Fig. 2). However, in fruit coated with DOO, the levels of APX remained similar to those found in fruit at harvest, what could be due to a H_2O_2 detoxification by different antioxidant mechanisms. CAT activity, another H_2O_2-scavenger enzyme, increased in all the dextrin coatings after 14 days of storage at 4 °C (Fig. 2). A similar behaviour has been reported in strawberries after applying different chitosan-based coatings, where this enzyme maintained high levels throughout storage at 5 °C (Wang and Gao, 2013). Gum arabic-coated persimmon fruit also exhibited substantially higher SOD, APX, and CAT activities (Saleem et al., 2020).

In zucchini fruit, different postharvest treatments have been reported to delay the appearance of CI and extend shelf-life during cold storage, inducing SOD, APX and CAT activities (Carvajal et al., 2015b; Palma et al., 2016); the use of dextrin could be a substitutive to these other treatments and this edible coating can be also supplemented with beneficial additives to increase antioxidant capacity. It has been described that caraway oil coating enriched with salicylic acid showed the higher activity of scavenger antioxidant enzymes including SOD, POD, and CAT in treated peppers under cold storage (Hanaei et al., 2022), and the combination of gum Arabic, oleic acid and cinnamon essential oil increased the antioxidant activity in guava fruit (Etemadipoor et al., 2020).

3.1.4. *Effect of a dextrin-based coating on non-enzymatic antioxidants defence compounds*

Non-enzymatic antioxidants, such as ascorbate, phenols or glutathione, are small molecules whose function is to supress the initiation, and break the propagation, of free radical chain reactions (Blokhina et al., 2003). Although ascorbate content increased with the storage period at 4 °C, its content was higher in dextrin-coated fruit, having DOO fruit the highest accumulation in ascorbate, 60% more than control fruit (Fig. 3).

This behaviour can be associated to what was described in the previous section, where the DOO coating maintained the APX activity at the same levels as freshly-harvested fruit. In zucchini fruit, it has been reported that ascorbate alleviates oxidative damage by scavenging radicals during postharvest storage (Carvajal et al., 2015b; Palma et al., 2016). The application of edible coatings to fruits of guava, persimmon and tomato also increased ascorbate levels during postharvest storage, contributing to diminish oxidative stress (Gurjar et al., 2018; Saleem et al., 2020; Suresh et al., 2022).

Phenolic compounds are secondary metabolites that contribute to the nutritional value of fruit and vegetables, and also increase their antioxidant contents (Molla et al., 2022), and also play a crucial role as part of the antioxidant defence system against cold stress during storage (Castro-Cegrí et al., 2023; Sayyari et al., 2011). These compounds also improve the nutraceutical quality of the fruit, as they are an important source of antioxidants (Cheng et al., 2020), of great importance for human health (Jideani et al., 2021; Swallah et al., 2020). During low-temperature storage of fruits, a decrease in total phenols has been related to an increase in CI, and treatments that alleviate CI also increase the total phenol content (Ngaffo Mekontso et al., 2021; Wang et al., 2019; Zhang et al., 2022). Fig. 3 shows that the level of phenolic compounds increased in control and in dextrin-coated fruit after 14 days of cold storage, but the levels were higher in the dextrin film complemented with both additives, which indicates a higher antioxidant capacity in these treatments. The most remarkable behaviour was observed for the DOO coating, as this treatment showed an important increase of total phenols. The use of edible coatings in strawberries and persimmon fruit also increased the total phenol content during postharvest storage (Mousavi et al., 2021; Saleem et al., 2020). Ca^{2+} cross-linked sodium alginate coating maintained higher total phenol and ascorbate content than the control fruit during cold storage in rose apple (Duong et al., 2022). As well known that chilling temperature stress could induce the accumulation of phenolics in fruit via the phenylpropanoid pathway (Sanchez-Ballesta et al., 2007).

3.1.5. *Effect of a dextrin-based coating on phenol-oxidizing enzymes*

Peroxidases (POD) and polyphenol oxidases (PPO) are the main enzymes responsible for fruit quality loss due to their activity in the degradation of phenolic compounds (Tomás-Barberán and Espín, 2001). For this reason, both enzymes have been reported to be involved in the development of several physiological disorders, such as CI (Trejo-Márquez et al., 2010). In our study, a 20% increase in POD activity was observed in control fruit at the end of storage, whereas all the dextrin-coated fruit maintained the POD activity during cold storage, with no increases observed (Fig. 4), so the amount of phenols in these fruits did not decrease.

In different fruits, POD activity has been shown to be induced by storage at low temperatures (Ali et al., 2022; Habibi et al., 2022; Wang, 1995). In mandarins, the chilling sensitivity of the peel of fruit correlated with the induction of the POD enzyme during storage (El-hilali et al., 2003). However, the use of chitosan coatings helped to inhibit this activity during postharvest storage (Shiekh et al., 2013). Other edible coatings also prevented the increased POD and PPO activities in strawberries kept at 4 °C, and consequently, the total phenolic compounds of the fruits treated with these films were preserved more effectively during cold stress (Mousavi et al., 2021). In our experiment, there were not differences between freshly-harvested fruit and control, D and DO-treated fruit after 14 days at 4 °C. However, dextrin complemented with olive oil was able to reduce this enzyme at the end of cold storage (Fig. 4), which is a very important result due to the ability of PPO to decrease the quality of the fruit. Mahmoudi et al. (2022) reported that chitosan-arginine nanoparticles application induced resistance to chilling injury in plum fruit by decreasing the activity of PPO enzyme. The behaviour of the POD and PPO enzymes correlates with the results obtained for the content of phenols described above. The response observed in the coatings is important to increase health-promoting properties of zucchini fruit while maintaining its postharvest quality during cold storage.

4. Conclusions

In conclusion, the dextrin-based coating was the most effective of all the edible polysaccharide coatings tested for maintaining the postharvest quality of zucchini fruit during cold storage. To enhance the properties of this polysaccharide coating in a second experiment, we used two additives obtained from olive trees, purified oleuropein and olive oil. The application of all dextrin coatings improved the storability of zucchini fruit at low temperature, maintaining fruit quality, increasing antioxidant defence, and diminishing oxidative stress. The addition of oleuropein and olive oil to the dextrin coating had some advantages related with fruit preservation, such as a higher induction of antioxidant enzymes, and a greater accumulation of ascorbate and total phenolics. The best results were obtained in the dextrin coating with olive oil, which were associated to the metabolism of phenolic compounds. These findings suggest that these types of preservation methods could be successfully incorporated to the distribution chain to extend postharvest life and enhance the overall quality of zucchini fruit.

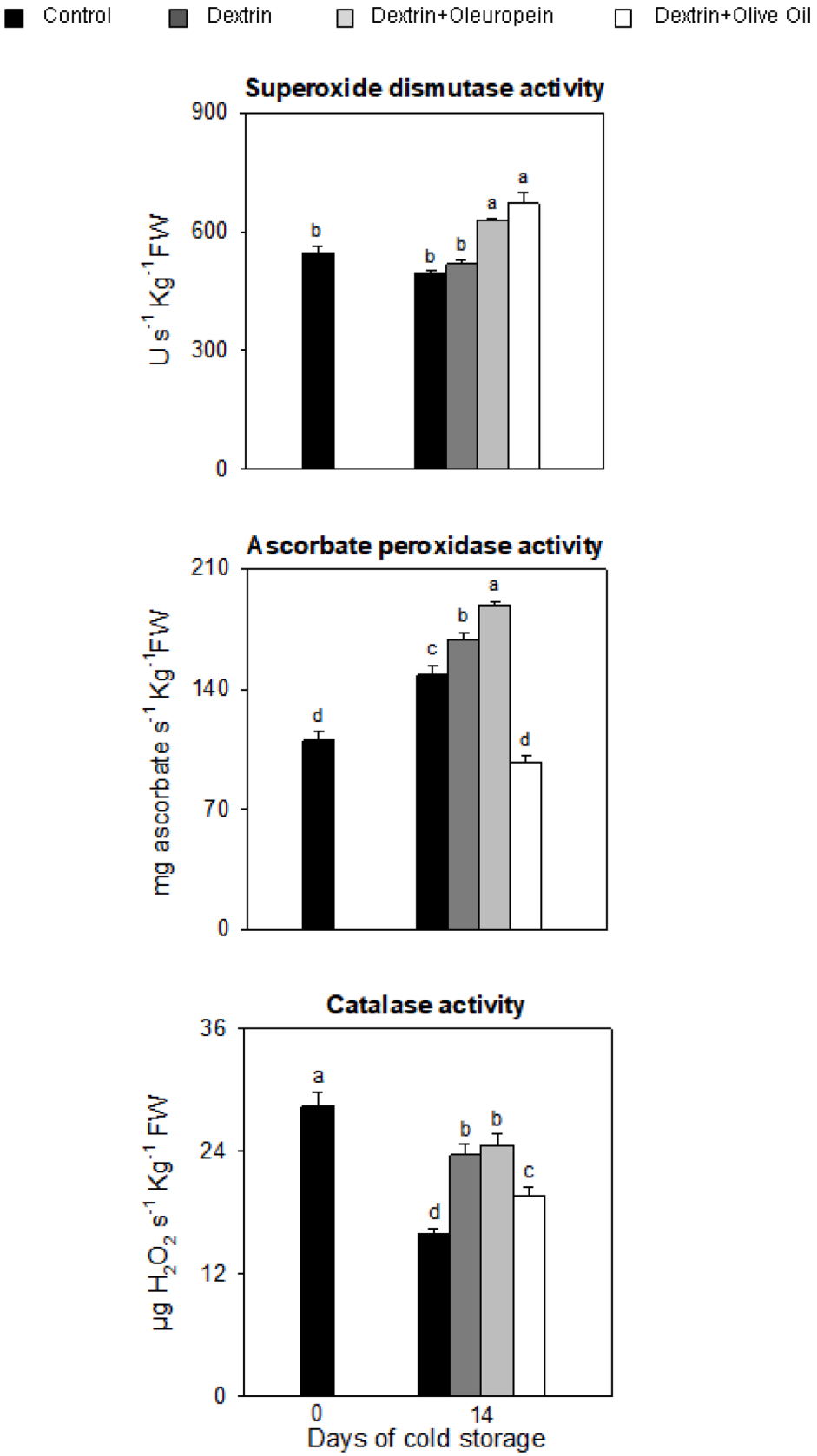

Fig. 2. Changes in the antioxidant enzymes superoxide dismutase, ascorbate peroxidase, and catalase of the exocarp of zucchini fruit control and coated with 1% dextrin (D), 1% dextrin plus oleuropein (DO) and 1% dextrin plus 0.2% olive oil stored during 14 days at 4 °C. Data presented are means ± SE of triplicate samples of six fruit each. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

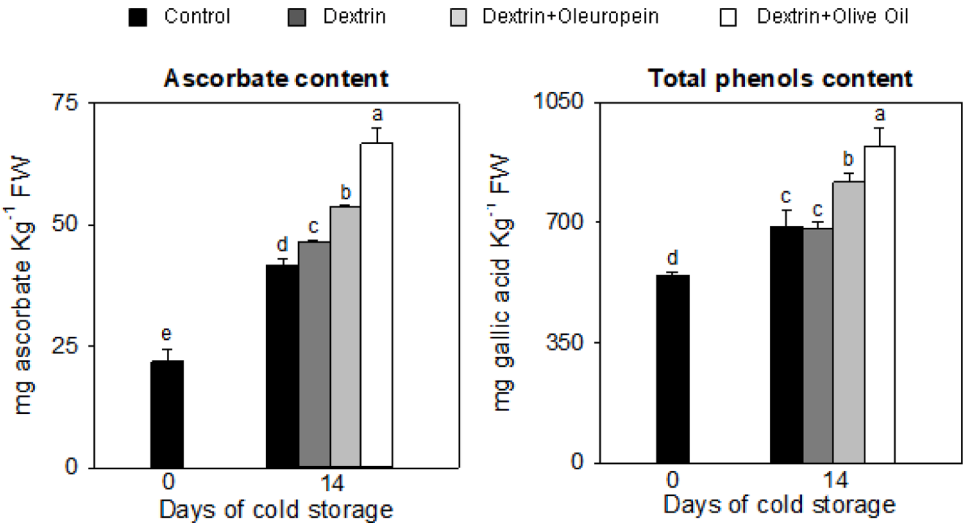

Fig. 3. Changes in ascorbate and total phenols content of the exocarp of zucchini fruit control and coated with 1% dextrin (D), 1% dextrin plus oleuropein (DO) and 1% dextrin plus 0.2% olive oil stored during 14 days at 4 °C. Data presented are means ± SE of triplicate samples of six fruit each. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

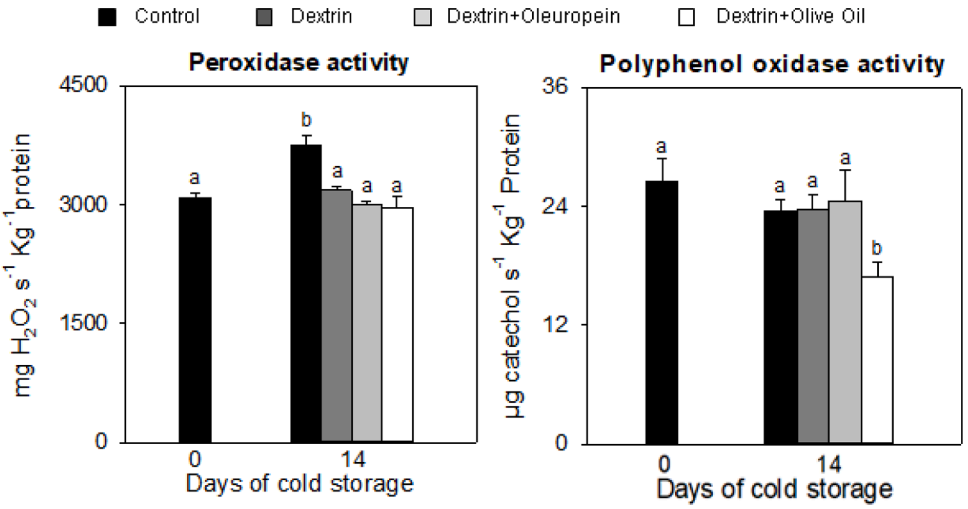

Fig. 4. Changes in phenol-oxidizing enzymes peroxidase and polyphenol oxidase of the exocarp of zucchini fruit control and coated with 1% dextrin (D), 1% dextrin plus oleuropein (DO) and 1% dextrin plus 0.2% olive oil stored during 14 days at 4 °C. Data presented are means ± SE of triplicate samples of six fruit each. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

CRediT authorship contribution statement

Alejandro Castro-Cegrí: Conceptualization, Methodology, Investigation, Writing – original draft. **Mariano Ortega-Muñoz:** Conceptualization, Methodology, Resources. **Sandra Sierra:** Investigation. **Fátima Carvajal:** Writing – original draft. **Francisco Santoyo-Gonzalez:** Resources. **Dolores Garrido:** Resources, Writing – review & editing. **Francisco Palma:** Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

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