Preservation of fresh-cut rocha pear using codium tomentosum extract

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Preservation of fresh-cut Rocha Pear using *Codium tomentosum* extract

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ABSTRACT

Rocha is a Portuguese pear cultivar with high economic importance in the Portuguese Western Region. Degradative processes following its manipulation can quickly lead to product rejection, especially when sold as a fresh-cut product. The efficacy of a marine-based edible coating to inhibit superficial browning development in fresh-cut Rocha pear slices was investigated over a storage period of 15 days. The aqueous extract of *Codium tomentosum*, an edible green seaweed, was incorporated in an edible coating (0.5 g 100 mL^{-1}) for fresh-cut Rocha pear. This novel treatment effect on the quality parameters of the pears was compared with a commercial coating currently used by industry and a control (dipping in deionised water). After 15 days storage at 4 °C, samples treated with the seaweed extract exhibited fewer colour changes and lower rates of superficial browning than control and commercial samples. Seaweed extract treatment was also observed to inhibit yeast and mould development, which may further contribute to shelf-life extension.

1. Introduction

Consumer acceptance of sustainable fresh fruits and vegetables depend on the freshness, quality, and convenience of the products available in the market. Fresh-cut fruits are a convenient option for daily life, in which the fruits are subjected to simple processes like disinfection, slicing and packaging (Augusto, Simões, Pedrosa, & Silva, 2016; Yousuf & Qadri, 2019). Given the processing steps involved (peeling, cutting, slicing), the shelf-life of fresh-cut fruits tends to be lower than the whole fruit, thus requiring additional preservation techniques (Ncama, Magwaza, Mditshwa, & Tesfay, 2018).

The use of edible coatings to extend keeping quality is economic and effective and widely used in the food industry (Ncama et al., 2018). Such coatings are applied by immersing the product into a coating solution or by spraying the solution, which, after drainage, generates a thin layer of surface coating. As the coating is consumed along with the product, its formulation must only contain a food-grade substance that doesn't interfere with the organoleptic quality of the fruit (Hassan, Ali, Chatha, Hussain, & Zia, 2018). The use of edible coating in fresh-cut fruits reduces senescence, controls moisture loss and gas exchange between the food and environment, thereby acting similar to modified atmosphere packaging. It is also possible to modify the coating formulation, depending on the application objectives and the type of fruit (Yousuf & Qadri, 2019).

One of the dominant impacts of processing fresh-cut fruits is the dramatic increase observed in the fruit metabolic rate, which consequently enhances the rates of enzymatic browning reactions (Dellarosa et al., 2016). This necessitates the application of anti-browning post-harvest treatments. Several anti-browning agents have been used to prevent tissue browning of fresh-cut fruits. Gomes, Fundo, Poças, and Almeida (2012) established the efficacy of calcium ascorbate solution with a pH of 7 as anti-browning in the case of fresh-cut Rocha pear and showed that browning was more marked under acidic conditions. In the case of fresh-cut 'Granny Smith' apples, a combination of citric acid (4.0–4.5%), ascorbic acid (3–4%) and *N*-acetyl-*L*-cysteine (1.5–2.0%) achieved a reduction in *Listeria monocytogenes and* at the same time maintained colour parameters over 21 days of storage at 4 °C (Fan, Sokorai, & Phillips, 2018). The application of hydrocolloid-based coating consisting of gellan, alginate and pectin combined with *N*-acetylcysteine and glutathione, to fresh-cut 'Flor de Invierno' pears controlled enzymatic browning, promoted microbial safety and reduced ethylene production ((Oms-Oliu, Soliva-Fortuny, & Martín-Belloso,

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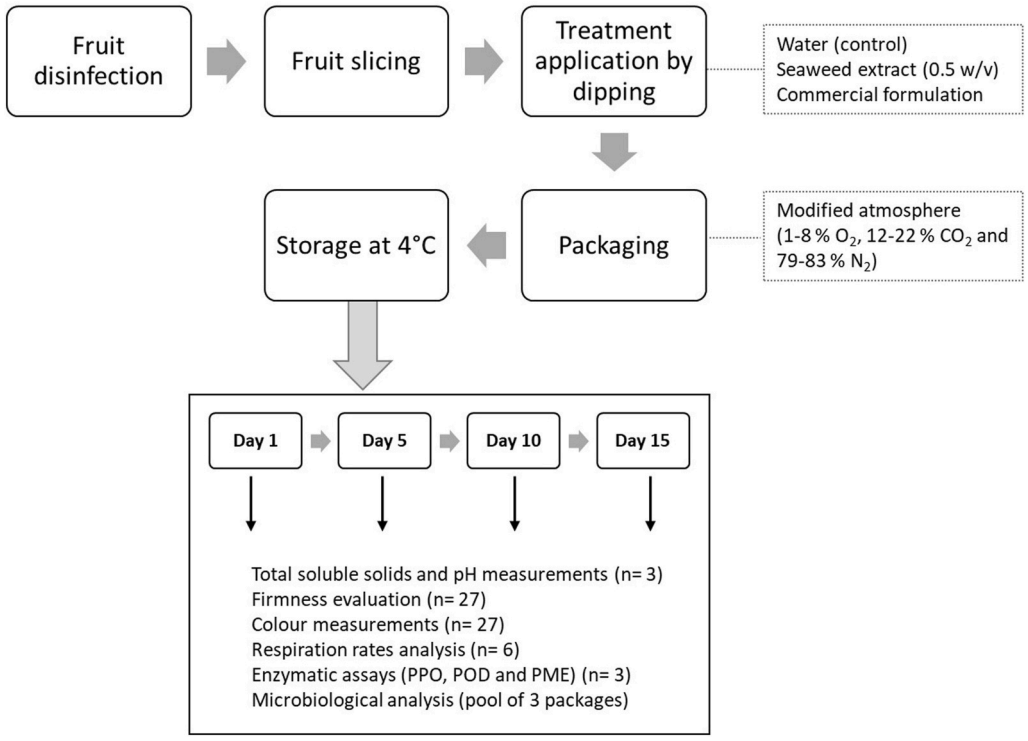

Fig. 1. Flowchart including the stages from fruit-slicing, coating and storage of samples, as well as the analysis performed each time.

2008)). Guerreiro, Gago, Faleiro, Miguel, and Antunes (2017) tested different formulations of alginate and pectin coatings incorporated with citral and eugenol on 'Bravo de Esmolfe' fresh-cut apple. These authors reported that coating with a solution containing 2% sodium alginate and 0.1% eugenol, followed by dipping in ascorbic acid (0.1%) resulted in improved anti-browning efficacy and sensory quality characteristics. Most of the substances used in the formulation of postharvest treatment solutions for extending the keeping quality of fresh-cut fruits are of synthetic origin and goes against current consumer trends, which favour the use of edible coatings and additives of natural origin (Mahajan et al., 2017). Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, and Martín-Belloso (2015) studied the effect of an edible coating formulated with nanoemulsions of sodium alginate and 0.1% (v/v) lemongrass essential oil (LEO) on the quality parameters of fresh-cut 'Fuji' apples. In the study, the authors observed higher *Escherichia coli* inactivation, slower microbial growth and lower browning in samples coated with a7 solution containing a low LEO concentration, instead of high LEO concentration (0.5 and 1% v/v). Whereas Hashemi, Mousavi, Ghaderi, and Ismail (2017) studied the use of a basil-seed gum coating containing the essential oil of *Origanum vulgare* to treat fresh-cut apricots, which demonstrated to extend fresh-cut apricots shelf-life by decreasing microbial spoilage, keeping the apricot quality-related features. Seaweeds have been used as a natural and renewable source of bioactive compounds possessing antimicrobial and antioxidant activities and are strong candidates to serve as natural additives for food preservation (Cian, Caballero, Sabbag, González, & Drago, 2014; Ummat, Sivagnanam, Rajauria, O'Donnell, & Tiwari, 2020). *Codium tomentosum* is a green edible seaweed, native to the North East of the Atlantic Ocean (Costa et al., 2015). Augusto et al. (2016) developed a coating solution containing 0.5% w/v of a hydroethanolic extract of *C. tomentosum* which delayed enzymatic browning of fresh-cut 'Fuji' apple stored under refrigerated conditions for 20 days at a laboratory scale. The authors observed a reduction in superficial browning development of 26% and attributed this observation to a significant reduction in the activities of enzymes like peroxidase and polyphenol oxidase caused by the coating. 'Rocha' pear (*Pyrus communis* L. cv Rocha) is an exclusive Portuguese pear variety, recognized as a Protected Denomination Origin and highly relevant to the economy of the Portuguese Western Region (Deuchande et al., 2016). In 2019, Portugal produced 15,3000 te of 'Rocha' pears of which 103,000 te – worth ninety thousand million USD– were exported (FAOSTAT, 2021). Due to specific climacteric and soil characteristics, 'Rocha' pear has distinct organoleptic characteristics and natural antioxidant profile, which differentiate it from other pear varieties (Coelho et al., 2019; Salta et al., 2010). 'Rocha' pear flesh is particularly sensitive to environmental changes, especially to temperature and light, which makes it more vulnerable to a high rate of superficial browning, particularly after slicing, which is the main obstacle for the successful commercialization of fresh-cut 'Rocha' pear. Pear cultivars which browns easily, exhibit cell membrane denaturation within the first 30 min after cutting (Li, Zhang, & Ge, 2017). With cell wall disruption, browning-related enzymes like peroxidase (POD) and polyphenol oxidases (PPO) leak and act on phenolic substrates which are present in significant concentrations in 'Rocha' pear (164.3 mg/100 g of fresh weight) (Salta et al., 2010). Given the pressing need for technological solutions to preserve fresh-cut 'Rocha' pear, especially for slowing down browning rates during storage, the present study aims to assess the use of the seaweed *Codium tomentosum* extract for coating fresh-cut Rocha pear.

2. Methods

2.1. Vegetable material

Campotec S.A. (Torres Vedras, Portugal), a local producer of fresh-cut fruits provided the 'Rocha' pear to be used in the experiments as well a commercial additive formulated with ascorbic acid that is currently used on their commercial products. Harvested pears were stored at room temperature (21 ± 3 °C) protected from light until use. The dried seaweed *Codium tomentosum* (particle size of 1.5 mm, dried at 25 °C) was obtained from ALGAplus (Ilhavo, Portugal).

2.2. Seaweed extract production

The extraction conditions were determined based on the work described by Augusto et al. (2016), with slight modifications. A ratio of 6.6% of seaweed to solvent (water) was stirred for 3 h at 15 °C to produce a batch of 5 L liquid extract using a solid-liquid extractor (Pilotdist SL5®, Meckenheim, Germany), which was subsequently freeze-dried (CoolSafe 55-4, LaboGene, Denmark) and stored protected from light at room temperature until use.

2.3. Fruit treatment and packaging

Before slicing, pears were sanitized with Amukina (Angelini pharma, Italy) following the manufacturer's instructions. A seaweed extract coating concentration of 0.5% (w/v) was used after investigating four coating concentrations in a preliminary study: 0.25%, 0.50%, 0.75% and 1% (w/v). The concentration of 0.50% (w/v) gave higher browning prevention efficacy following storage for 9 days at 4 °C. An automatic slicer (Turatti, Italy) was used to produce pears slices, each weighing *ca.* 11 g. A 0.5% (w/v) coating solution of the seaweed extract was prepared, and 100 g of fruit slices were immersed in 200 mL of the solution at 4 °C for 1 min. Likewise, a similar solution of the commercial ascorbic acid based formulation was prepared and the same weight of cut fruit slices were similarly immersed in this solution. Control samples of pear slices were obtained by simply dipping the slices in deionised water. Comparisons had to be made under similar conditions and all factors had to be maintained – which included the water content of the fresh-cut samples. Therefore deionised water was used as a dipping solution. After coating, the slices (86.01 ± 9.65 g) were packaged in 200 cm^3 plastic film bags (Pigmea, Jaén, Spain) under modified atmosphere conditions (1–8% oxygen, 12–22% carbon dioxide and 79–83% nitrogen at 8 °C) using an Ulma packaging system (Oñati, Spain).

A total of 48 packages were prepared with each coating solution (seaweed extract and commercial coating solutions) and control and stored at 4 °C for 15 days. Every 5 days, 12 packages were used for assessing the fruit quality parameters, enzymatic assays and microbiology enumeration. A flowchart comprising fruit treatment and analysis performed during storage is given in Fig. 1.

2.4. Physicochemical quality evaluation

Total soluble solids (TSS) were determined as the degree of brix (°Brix) using a digital refractometer (RFM340+, Paragon Scientific, Liverpool, UK). pH was directly measured in samples according to the procedure described by Augusto et al. (2016). Tests were performed using one slice per package and 3 packages per treatment (n = 3), per sampling time.

The firmness of samples was assessed by a compression test using a texture analyser TA.XT.Plus (Stable Micro Systems, Surrey, England). Firmness was considered to be the maximum force measured when a 5 mm cylindrical probe penetrated a depth of 5 mm at a speed of 1.5 mm s^{-1} (Augusto et al., 2016). The test was performed using three slices per package and 3 packages per treatment. Further, each slice was pierced in three different areas (one central and two near the edges, thereby giving 27 readings for each storage conditions.

Colour was assessed using CIELAB system with a portable colourimeter (Konica Minolta, CR 400, Japan) as described by Augusto et al. (2016). The results were expressed as colour changes (ΔE*) and browning index (BI). The Euclidean distance of two points (ΔE*) was calculated between a fresh-cut sample without treatment on day 0 and the day of analysis according to the equation described by Lante, Tinello, and Nicoletto (2016). The browning index, BI, was estimated as Augusto et al. (2016):

$$BI = \frac{x - 0.31}{0.172} \times 100 \quad (1)$$

where

$$x = \frac{a + 1.75L}{5.645L + a - 3.012b} \quad (2)$$

Colour evaluation was conducted using three slices per package and 3 packages per treatment. Here too, the colour was measured at three locations on each slice, one at the centre and two near the edges of the slices, giving 27 sets of readings for each storage conditions.

2.5. Headspace gases

An OxyBaby® 6.0i (Witt, Witten, Germany) gas analyser was used to measure the percentage of oxygen and carbon dioxide inside the packaging, which enabled calculation of the respiration rates of the slices during storage, as described by Dellarosa et al. (2016). Six packages per treatment (n = 6) were analysed per sampling time.

2.6. Enzymatic assays

The extraction protocol of peroxidase (POD) and polyphenol oxidase (PPO) enzymes was performed as described by Augusto et al. (2016). The supernatant was divided in aliquots for the enzymatic assays and Bradford method for protein quantification (Bradford, 1976). The determination of the enzymatic activity of both enzymes followed the methodology proposed by the same authors, adapted for a reaction volume of 300 μL in a 96-multi-well plate. POD activity was assessed at 470 nm for 10 min, and PPO activity at 400 nm over 2 min of reaction. Results were expressed as U mg^{-1} protein g^{-1} of fresh weight (FW).

For pectin methylesterase (PME) extraction, the conditions for PPO and POD extraction, described above, were replicated but using 1.5 M of NaCl with 2.5 w/v of polyvinylpyrrolidone (PVP) as extraction buffer. The determination of PME activity was adapted from Liu et al. (2016) and Delgado-Reyes, Fernández Romero, and Luque De Castro (2001). To a 96-multi-well plate were added 15 μL of 0.01% bromothymol blue (in 0.003 M sodium phosphate buffer, pH 7.5), 235 μL of 5 g L^{-1} citrus pectin (pH 7.5) and 50 μL of the extracted sample (pH 7.5). The reaction was conducted for 4 min at 35 °C and followed by spectrophotometry at 610 nm. Results were expressed as U mg^{-1} protein g^{-1} of FW. Enzymatic activities were evaluated by pooling three slices per package and 3 packages per treatment (n = 3), per sampling time.

2.7. Microbiological analysis

The microbiological analysis followed the protocols approved and published by the International Organization for Standardization (ISO). Sample homogenization and decimal dilutions were made with buffered peptone water (ISO 6887-4: 2017). The ISO 4883-2: 2013 was followed for the enumeration of microorganisms at 30 °C and 4 °C, and the ISO 21527-1: 2004 for the enumeration of yeasts and moulds. A pool of three packages per treatment per sampling time was used for homogenization and further analysis. Even though the bacterial count exceeded safety limits after 10 days of storage at 30 °C and 4 °C, the evaluation of microbial counts and assessment of browning continued until day 15, in order to establish the relative efficacies of the seaweed extract and the commercial coating.

2.8. Statistical data analysis

For the data analysis, the influence of coating solution and storage time on the samples coated with the seaweed extract or the commercial solution were evaluated. R statistical and programming environment (R Development Core Team, 2012) was used with the *nlme* package (Pinheiro et al., 2017), to test independent regression models for each dependent variable (TSS, pH, firmness, ΔE, BI, $RRCO_2$, POD, PPO and PME). When necessary, a Generalized Least Squares (GLS) procedure

Table 1
Summary of regression models (GLS) to examine the effects of coating solution (treatment) and storage time (day) in the response variables for shelf-life quality and enzymes activity. For each model, are listed the main terms, variance-covariate, degrees of freedom (d.f.), likelihood ratio (L-ratio) and level of significance (p-value).

Dependent Variable	Significant terms/ Main terms	Variance-covariate	d. f.	L-ratio	p-value
TSS	Day	–	9	23.35	0.0055
pH	Treatment x Day	Day	6	20.54	0.0022
Firmness	Treatment x Day	–	6	27.06	1e-04
ΔE	Treatment x Day	–	6	33.72	<0.0001
BI	Treatment x Day	–	6	38.19	<0.0001
$RRCO_2$	Treatment x Day	Treatment x Day	6	23.92	5e-04
POD	Treatment x Day	Day	6	22.38	0.001
PPO	Treatment x Day	Treatment	6	27.54	1e-04
PME	Treatment x Day	Day	6	26.82	2e-04

TSS: total soluble solids; ΔE: colour differences; BI: browning index; $RRCO_2$: respiration rate; POD: peroxidade; PPO: polyphenol oxidase; PME: pectin methylesterase.

was used with the appropriate variance covariate structure (Pinheiro et al., 2017). This avoided data transformation by allowing residual spread to vary with the explanatory variables. CANOCO version 4.5 package was used for the Principal Component Analysis (PCA) design.

3. Results and discussion

3.1. Physicochemical quality evaluation and colour parameters

The present work is the first study where a 100% aqueous extract of *Codium tomentosum* is used for coating fresh-cut fruits. However, in the studies of Augusto et al. (2016, 2018) a hydroethanolic mixture containing 75/25 (v/v) of water to ethanol was used for *C. tomentosum* extracts production. Water was used as extraction solvent because the functional components responsible for lowering browning is water soluble. If organic solvent was used, then its application to fresh-cut fruits will require additional safety compliances (Directive 2009/32/EC).

Total soluble solids (TSS) and pH are two indicators regularly monitored to assess product quality over storage. TSS and pH values of fresh-cut pear slices at day 0 were 10.91 ± 1.31% and of 4.66 ± 0.30 respectively, values in line with those reported in the literature, where TSS values ranged from 10.40% to 11.58% (Carra et al., 2018; Saquet & Almeida, 2017) and 4.47 to 4.79 (Gomes et al., 2012). All these values were within the limit of 10% of higher values considered for product quality acceptance (Saquet, 2019).

The statistical model confirmed that there were no differences

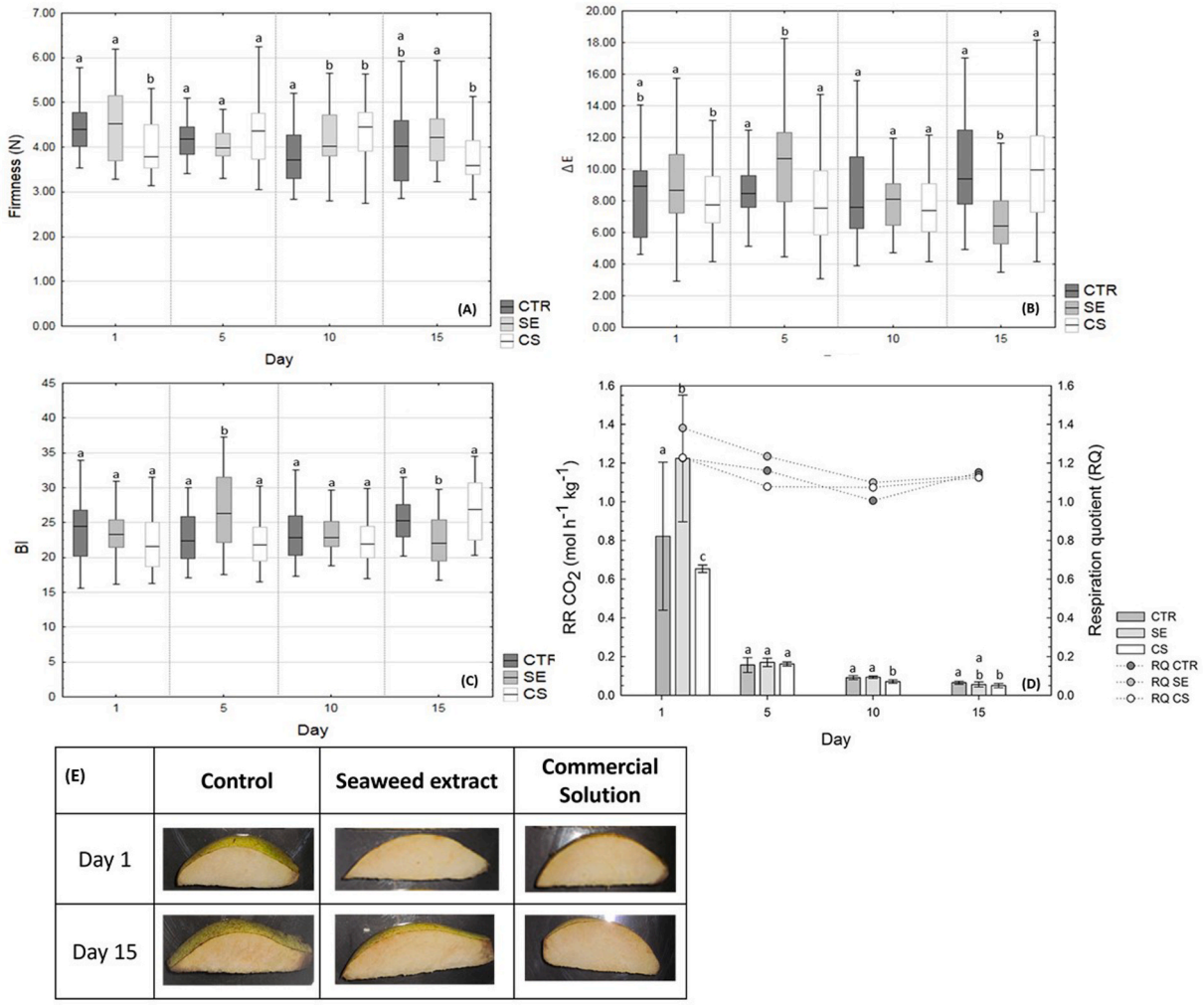

Fig. 2. Boxplots of firmness (N) **(A)**, Colour differences (ΔE) **(B)**, Browning index (BI) **(C)** and Respiration rate expressed as the production of carbon dioxide (RR CO_2), respiration quotient **(D)** and visual photographs **(E)** of fresh cut pears treated with seaweed extract (SE), commercial solution (CS) and control (CTR), and stored for 15 days at 4 °C. In (D), vertical bars represent mean ± standard error. On each day, boxplots or bars with different letters represent significantly different values (ANOVA, GLS, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

between treatments in TSS during the experimental period (Table 1). Regarding pH, there was a significant effect of the interaction between treatment and days of storage (Table 1). However, the observed decrease in pH values was residual with no consequences on the physicochemical profile of the fresh-cut pear slices.

Fruit firmness is another quality attribute determined. It has a high impact on the shelf-life of fresh-cut fruits, working as an indicator of fruit freshness, and internal attributes which can potentially influence a consumer's purchasing intention (Belay, Caleb, & Opara, 2019; Li et al., 2020). Firmness values for all treatments during storage are presented in Fig. 2A.

Firmness was influenced by the interaction coating solution *x* storage time (Table 1), and the variable with a higher impact on its values was storage time (L-ratio = 35.278, df = 9, p = 1e-04), with a smaller contribution to the statistical model of the coating solution (L-ratio = 30.998, df = 8, p = 1e-04). A slight difference, although significant, was detected at day 1, between commercial solution and control (t = 2.125, p = 0.034), and seaweed extract coated samples (t = −2.652, p = 0.008), with the commercial coated samples presenting lower values. Over storage time, variations were detected namely on days 10 and 15. At day 10, control samples showed lower values - around 11.5% and 16.5% - than seaweed extract (t = −2.486, p = 0.013) and commercial solution (t = −3.465, p = 0.001) samples. Although, at the end of the study, control samples had similar values to samples coated with the seaweed extract (t = −1.264, p = 0.207) and commercial solution (t = 1.719, p = 0.087). However, when comparing coating solutions at day 15, it is possible to observe that seaweed extract samples had 10% higher firmness values than those treated with the commercial solution (t = −2.957, p = 0.003), thereby indicating efficacy of the seaweed extract to preserve fresh-cut pear slices firmness during cold storage. A hydroethanolic extract *of C. tomentosum* was also reported to efficiently preserve firmness of fresh-cut apple slices, after 20 days at 4 °C (Augusto et al., 2016). The firmness maintenance of the fresh-cut pear slices coated with the seaweed extract was also confirmed by the unchanged firmness between days 1 and 15 of storage (t = 1.363, p = 0.174) (Supplementary data, Table S1). This effect may be due to the incorporation of bioactive compounds from the seaweed which enhance cell integrity, lower component leakage from the cell and prevent flesh softening (Fundo et al., 2016). In the case of *C. tomentosum* extract which is poor in antioxidant molecules (Augusto et al., 2016; Silva et al., 2020), the cell wall protection by the extract may be specifically related with the presence of sulphated polysaccharide in the extract composition (Augusto et al., 2018). Although with lower values of firmness, commercial coated samples also did not show a significant decrease at the end of the experiment (t = 1.363, p = 0.174). At the end of the study, a loss of about 6% in firmness was observed in control (t = 2.117, p = 0.035) (Supplementary data, Table S1).

Colour changes are a good indicator of the freshness of fresh-cut fruits, the emergence of brown colour is commonly associated with the decrease in freshness and quality, which also represents the limiting factor for consumer acceptability (Belay et al., 2019). The determined colour parameters – colour differences (ΔE) and browning index (BI) are presented in Fig. 2B and C, respectively. As observed in the case of the quality parameters presented above, the variable that influences colour results the most is storage time (ΔE: L-ratio = 39.009, df = 9, p < 0.0001; BI: L-ratio = 46.539, df = 9, p < 0.0001), when compared with coating solution (ΔE: L-ratio = 34.907, df = 8, p < 0.0001; BI: L-ratio = 39.176, df = 8, p < 0.0001). This is an expected result, since shelf-life decays significantly over time even when preservatives such as ascorbic and citric acids, among others, are applied (Zheng, Liu, Liu, Liu, & Zheng, 2019). Determining colour changes (ΔE) in fresh-cut fruits is a simple measure of the ability of a coating to preserve colour: higher values of ΔE usually denote a lower product efficacy. In the present work, it was possible to observe that samples coated with seaweed extract had lower colour differences than control (t = 3.751, p < 0.0001) and commercial solution treatment (t = 4.325, p < 0.0001) (Fig. 2B), after 15 days of storage. The visual photograph of the fruit can be observed in Fig. 2E. In a previous study, it was demonstrated that the hydroethanolic extract of *C. tomentosum* was also able to prevent colour changes in fresh-cut apples (Augusto et al., 2016). The results observed in the present work indicate a potential functionality of the extract in delaying colour changes in fresh-cut pear, widening the application of *C. tomentosum* extract to fresh-cut fruits. The initial variations in ΔE, with statistical significance, namely between commercial solution and seaweed extract coated samples observed at day 1 (t = −1.759, p = 0.08), can be explained by the instant browning which occurs immediately after the cutting process (Zheng et al., 2019). The browning index results (Fig. 2C), follow the trend reported for ΔE. On the last day of the experiment, day 15, the seaweed extract treated samples had a BI value 15–16% lower than control (t = 2.235, p = 0.026) and commercial solution (t = 4.075, p = 0.00) samples. Besides, the samples treated with the seaweed extract had a similar BI value at days 1 and 15 (t = 0.687, p = 0.493), while samples coated with commercial solution presented an increase of about 20% in BI values over storage time (t = −4.131, p = 0.00) (Supplementary data, Table S2). In pears, especially in Rocha pear cultivar, the high polyphenol content makes it susceptible to oxidation and leads to rapid and severe browning after cutting (Gomes, Vieira, Fundo, & Almeida, 2014; Zheng et al., 2019). This makes it challenging to formulate a coating with high efficacy to reduce browning. Nevertheless, as reported above, seaweed extract treatment maintains colour after 15 days of storage. The browning inhibition efficacy may be attributed to the presence of polysaccharides in the extract composition (Augusto et al., 2018), which, once in contact with pear slices, forms a protective barrier between the cells and the environment. This barrier reduces oxygen gas exchange, and lowers the oxidative stress and the levels of peroxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which are necessary for browning development (Wang, Li, Li, Li, & Luo, 2021).

3.2. Respiration rates

Like all other fruits, the respiration rate of fresh-cut pear slices increases significantly in response to the wounding of tissues which is caused during slicing. The use of modified atmospheres packaging along with other postharvest techniques is employed by industry to control the effects of high respiration (Gomes et al., 2014). The respiration rates expressed as CO_2 production and the respiration quotient results are presented in Fig. 2D. There were interactive effects of coating solution and storage time on CO_2 production (Table 1), where days of storage was the variable with a higher influence on the respiration rates (L-ratio = 144.583, df = 9, p < 0.0001, in comparison with coating solution: L-ratio = 34.672, df = 8, p < 0.0001). With time, the composition of the modified atmosphere changes as consequence of gas exchange and respiration rates, which leads to a sharp decrease in CO_2 production in all samples after 15 days of storage (Fig. 2D).

Since respiration quotient values ranged between 1.00 and 1.38 (Fig. 2), it is possible to hypothesise that fresh-cut pear slices used organic acids as the major respiration substrate (Fonseca, Oliveira, & Brecht, 2002), a fact also observed by Gomes et al. (2012) in a study with fresh-cut Rocha pear.

3.3. Enzymatic activities

As mentioned before, browning is one of the driver factors determining consumer acceptance in the case of fresh-cut fruits. The two main enzymes responsible for this phenomena are polyphenol oxidase (PPO) and peroxidase (POD) (Kou et al., 2015). These enzymes accelerate pear slices deterioration, leading to the advent of browning spots on the surface. Therefore, it is essential to monitor POD and PPO activities during storage.

POD activity suffered the interactive effect of coating solution *x* storage time (Table 1 and Fig. 3A). In relation to the results obtained for POD activity, the variable which predominantly contributed to these

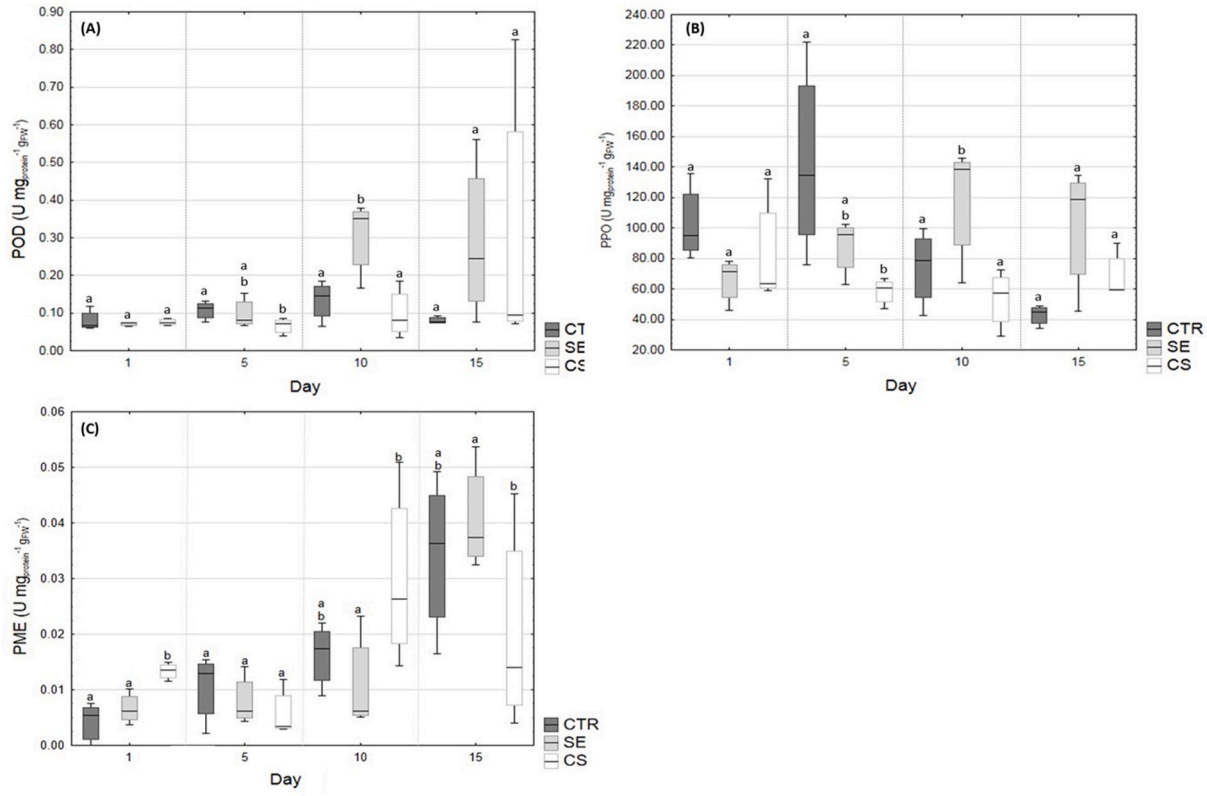

Fig. 3. The activities of **(A)** Peroxidase (POD), **(B)** Polyphenol oxidase (PPO), and **(C)** Pectin methylesterase (PME) of fresh-cut pears treated with seaweed extract (SE), commercial solution (CS) and control (CTR), and stored for 15 days at 4 °C. On each day, boxplots with different letters are significantly different (ANOVA, GLS, $P < 0.05$).

results was storage day (L-ratio = 34.993, df = 9, p = 1e-04, vs coating solution: L-ratio = 23.170, df = 8, p = 0.003). It is possible to observe that, on the first day of storage, all analysed samples had similar values of enzymatic activity (Fig. 3A, $p > 0.05$, Supplementary data, Table S3). However, after 5 days, the samples coated with the commercial solution presented lower values of POD activity when compared with control (T = 2.243, p = 0.034), and no significant differences were found between commercially coated samples and those coated with seaweed extract (t = −1.48, p = 0.152). At day 10, the samples coated with the seaweed extract solution gave a higher POD activity when compared to CTR (t = −3.784, p = 0.001) and CS samples (t = −4.676, $p < 0.0001$). In an earlier study, Augusto et al. (2016) found that the POD activity of apple slices was lower in the control sample than in slices coated with a similar seaweed extract. In the present case, after 15 days of storage, the commercial coating and seaweed extract coating had the same effect on POD reduction ($p > 0.05$, Supplementary data, Table S3).

In the case of PPO activity, the coating solution employed was the variable with the highest influence on the results despite there being significant interaction with storage period (Table 1) (L-ratio = 38.072, df = 8, $p < 0.0001$, vs storage time: L-ratio = 28.122, df = 9, p = 9e-04). On day 1, no differences between treatments were detected ($p > 0.05$, Supplementary data, Table S4). Only after 5 days, the changes in PPO activity were detected (Fig. 3B). The control showed higher values of PPO when compared with commercial solution treatment (t = 2.243, p = 0.034). No differences in PPO activity were detected between seaweed extract and commercial solution coated samples, indicating a similar effect on PPO activity (t = −1.48, p = 0.152). After day 10, the seaweed extract treatment showed lower efficacy in respect of decreasing the PPO activity when compared with the commercial solution treatment (t = −4.676, p = 0.000) and control (t = −3.784, p = 0.001). Also, on day 15, there were no statistical differences between the coating solutions ($P > 0.01$, Supplementary data, Table S4), suggesting comparable efficacies of the seaweed and commercial extracts.

It is interesting to note that the enzyme activities of PPO and POD are greater after 15 days of storage than after day 1 for the same treatment, a trend that is not in accordance with colour results (Fig. 2B and C). In terms of colour parameters, seaweed extract application led to slower browning over time, and these results were not correlated with PPO and POD activities. Despite the significance of these enzymes in pear browning processes, several authors have noted that PPO and POD activities are not the only key factors for brown colour development in sliced pear (Gomes et al., 2014; Li et al., 2017; Liao et al., 2020). Maillard reaction is known to cause significant levels of non-enzymatic browning, especially in high sugar containing fruits (Paravisini & Peterson, 2018). The results obtained in this work on 'Rocha' Pear also seem consistent with these authors. However, more studies on the mechanism of action of PPO and POD are needed to understand the efficacy of the coatings.

With storage time, several cell wall-related enzymes, such as pectin methylesterases (PME), have an important role in fruit postharvest softening by regulating cellular wall degradation (Liu et al., 2016), including the hydrolysis of the galacturonic acid, a major component of pectin, inducing pectin solubilisation and consequently firmness loss (L'Enfant et al., 2015).

Fig. 3C shows that the PME activity increases with storage time for all samples. The statistical model showed a significant interaction between coating solution and storage days (Table 1), with a higher contribution of (L-ratio = 45.047, df = 9, $p < 0.0001$, vs coating solution: L-ratio = 33.004, df = 8, p = 1e-04). After day 1, the samples coated with the commercial solution had higher values of PME when compared with control (t = −4.914, p = 0.00) and seaweed extract treatment (t = 3.762, p = 0.001). However, at day 5 a different trend was observed, with the increase of PME activity in control and seaweed extract samples, although without significant differences ($p > 0.05$,

Table 2
Total mesophilic bacteria count (log CFU g^{-1}), psychotropic bacteria (log CFU g^{-1}), yeasts, and moulds (log CFU g^{-1}) of fresh-cut pears subjected to different treatments and stored for 15 days at 4 °C.

Storage day	Control	Seaweed extract	Commercial Solution
Mesophilic bacteria (log CFU g^{-1})			
1	4.35	4.20	4.02
5	5.24	4.86	4.45
10	7.06	7.39	7.01
Psychrotrophic bacteria (log CFU g^{-1})			
1	3.97	3.91	3.73
5	5.3	5.69	5.40
10	7.27	7.35	6.94
Yeasts and moulds (log CFU g^{-1})			
1	2.04	2.02	2.00
5	N.D.	N.D.	N.D.
10	N.D.	N.D.	2.10
15	3.75	1.80	3.10

N.D. Not detected.

Supplementary data, Table S5). After 10 days a switch in PME was observed: samples treated with seaweed extract had significantly lower values than those treated with the commercial solution ($t = 2.778$, $p = 0.01$), and no differences to control ($t = 0.977$, $p = 0.338$). At the end of the storage period, although significant, the seaweed extract coated samples had a slightly higher value of PME than the samples treated with the commercial solution ($t = -2.30$, $p = 0.03$). Despite the initial PME activity in samples treated with commercial solution, after 15 days the values remained stable ($t = -0.805$, $p = 0.429$) (Supplementary data, Table S5), and in contrast, the control and seaweed extracted samples gave significantly higher PME activity [683% ($t = 4.563$, $p = 0.00$) and 511% ($t = -5.026$, $p = 0.00$), respectively] (Fig. 3C; Supplementary data, Table S5).

3.4. Microbiological analysis

After 10 days of storage, the mesophilic and psychrotrophic bacteria counts of all samples exceeded the legal criteria (i.e. 6 log CFU g^{-1} recommended by the Portuguese government (Santos, Correia, Cunha, Saraiva, & Novais, 2005)) (Table 2). Similar results were obtained by Gomes et al. (2012) in a study of fresh-cut Rocha pear packaged under modified atmosphere. Neither the seaweed extract nor the commercial coating solution showed activity against mesophilic and psychrotrophic bacteria, although further studies are needed to detect the efficacy of the seaweed extract against specific pathogenic microorganisms, e.g. *Escherichia coli*, *Salmonella enterica* and *Listeria* spp., especially since Rocha pear is known to be a good substrate for the survival of pathogenic bacteria (Graça, Santo, Quintas, & Nunes, 2017).

In relation to yeasts and moulds, all the samples analysed had counts below the recommended threshold limits (3–5 log CFU g^{-1}) (Santos et al., 2005) (Table 2). It is also worth highlighting that the seaweed extract treated samples showed lower counts of yeasts and moulds than control and commercial solution treatment, which suggests that the seaweed extract may possess antifungal activities not yet reported. Only a few studies have reported on antimicrobial activity of this seaweed extracts, however using organic solvents in the extractions (Silva et al., 2020). In an unpublished work conducted by the authors of the present study, a solution of *C. tomentosum* extract applied to fresh-cut apples showed inhibitory effect in the case of yeasts and moulds growth over 20 days of refrigerated storage. Being these the first reports regarding *C. tomentosum* aqueous extract antifungal activity, more studies are necessary to identify the compounds responsible and then access the mechanism of action behind this action.

3.5. Principal component analysis

Principal component analysis (PCA) was used to assess how the measured variables influences samples to be similar to, or when or how they differ from each other (Sun, Liu, Li, Wu, & Zhu, 2016). PCA was applied to evaluate qualitative differences between the two coating solutions and control samples. The principal component 1 (PC 1) and 2 (PC 2) accounted for 61.2% of the total variance, with 41.0% being explained by axis 1 (PC 1) (Fig. 4).

After days 1 and 5, the coated and control samples showed similar

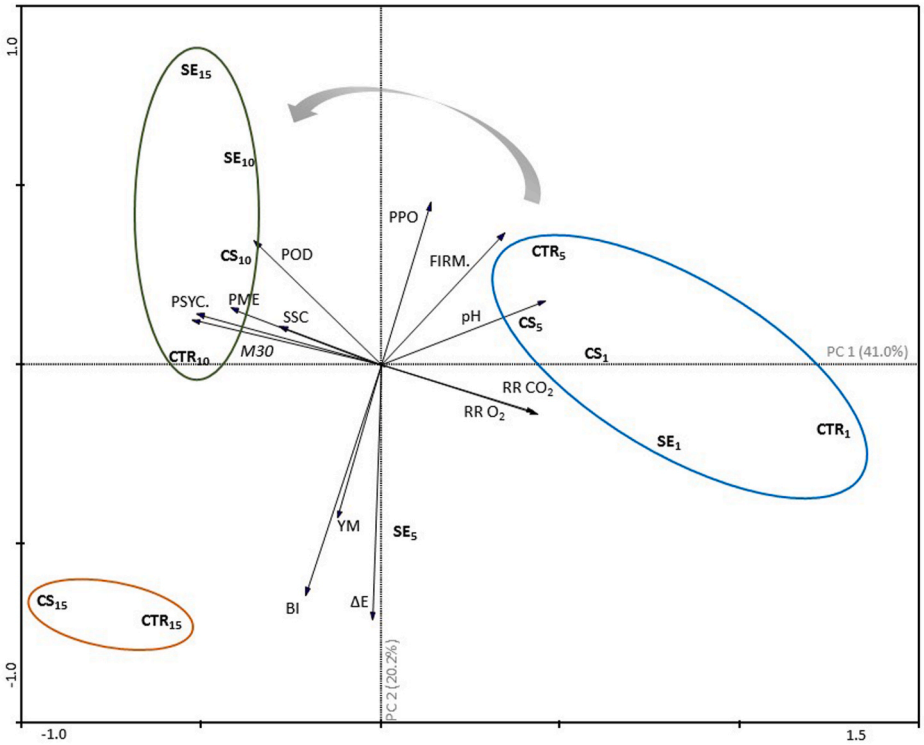

Fig. 4. Biplot from principal component analysis integrating all physical and chemical parameters, enzymatic activities and microbiological analysis in fresh-cut pears treated with seaweed extract (SE), commercial solution (CS) and control (CTR) and stored at 4 °C for 1, 5, 10 and 15 days. Firm.- firmness, SSC- soluble solids concentration, RR CO_2- CO_2 production, RR O_2- O_2 consumption, ΔE-colour differences, BI- browning index, PPO- Polyphenol oxidase, POD- Peroxidase, PME- Pectin methylesterase, YM-yeasts and moulds, M30-mesophilic bacteria, PSYC.- psychrotrophic bacteria. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

results and formed a cluster (Blue line, Fig. 4). In this cluster, respiration rates (RRO_2 and $RRCO_2$), pH, firmness and polyphenol oxidase activity are the main vectors influencing the results. Although there are similarities between samples at day 5, the results of the seaweed extract coated samples were mostly differentiated by colour changes and browning index.

A second cluster emerged at day 10 (Green cluster, Fig. 4), where the main vectors were peroxidase and pectin methylesterase enzymes, total soluble solids, mesophilic and psychrotrophic bacteria. Considering the proximity of these vectors to the control and commercial solution treatment at day 10, it is possible to conclude that the results of these samples were markedly influenced by those vectors. And looking for the samples coated with the seaweed extract solution, at days 10 and 15, and included in the same cluster, it is also possible to observe a relationship between the vectors mentioned above and the sample results. Also, the proximity of the seaweed extract sample vectors at days 10 and 15, and included in the same cluster, may represent lesser changes in the physicochemical properties at the end of the storage period.

A third cluster was defined, but only including control and commercial samples at day 15 (Orange line, Fig. 4). The results of control and commercial solution treatment after 15 days of storage were mainly influenced by the high values of the browning index and colour changes. This influence in control and commercial sample results also evidence the superficial browning development observed after the 15 days of storage, while the samples coated with the seaweed extract (SE_{15}) were marginally influenced by colour parameters (observed by the 90° angle with colour vectors), justifying the browning delay of the seaweed coated samples.

4. Conclusions

The efficacy of the aqueous extract of *C. tomentosum* in delaying the superficial browning of fresh-cut Rocha pear was evaluated. Samples treated with the seaweed extract showed considerable stability in terms of total soluble solids and firmness, after 5 days of storage. Samples treated with this solution showed lower colour change and browning index than control and commercial samples. Samples treated with the seaweed extract also showed lower development of yeasts and moulds, indicating some antimicrobial activity. The anti-browning and anti-fungal actions, especially their underpinning mechanisms require further research attention.

CRediT authorship contribution statement

Ana Augusto: Conceptualization, Methodology, Writing – original draft. **Andreia Miranda:** Methodology. **Daniel Crespo:** Formal analysis. **Maria J. Campos:** Validation. **Délio Raimundo:** Validation, Resources. **Rui Pedrosa:** Supervision, Funding acquisition. **Geoffrey Mitchell:** Supervision. **Keshavan Niranjan:** Supervision, Writing – review & editing. **Susana F.J. Silva:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

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.

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Appendix A. Supplementary data

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