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A pilot plant scale testing of the application of seaweed-based natural coating and modified atmosphere packaging for shelf-life extension of fresh-cut apple

Ana Augusto[a,b,c*], Andreia Miranda[a], Leonor Costa[d], Joaquina Pinheiro[a], Maria J. Campos[a], Délio Raimundo[e], Rui Pedrosa[a], Geoffrey Mitchell[c], Keshavan Niranjan[b], Susana F.J. Silva[a]

[a]MARE - Centro de Ciências do Mar e do Ambiente, ESTM, Politécnico de Leiria, 2520-641 Peniche, Portugal; [b] Department of Food and Nutritional Sciences, University of Reading, Whiteknights, RG6 6AP, Reading, United Kingdom; [c]Centre for Rapid and Sustainable Product Development (CDRsp), Politécnico de Leiria, 2430-028 Marinha Grande; m; [d] iBET – Instituto de Biologia Experimental e Tecnológica, 2781-901 Oeiras, Portugal; [e] Campotec S.A., 2560-393 Silveira, Torres Vedras, Portugal

*Corresponding author: E-mail address: ana.l.augusto@ipleiria.pt (A. Augusto). ORCID ID: 0000-0001-8561-6848

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Abstract

Codium tomentosum hydroethanolic extract was obtained using a pilot solid-liquid extractor to validate the anti-browning functionality of the extract under industrial conditions. Fresh-cut apple slices were coated by immersion in: 1) a seaweed extract solution (0.5 % w/v) and 2) a commercial coating, and the two sets of samples were compared with a control (immersion in water). Packaged samples were stored, under ambient and modified atmosphere conditions at 4˚C. After 30 days of storage, the samples which were coated with the seaweed extract and packaged under modified atmosphere, demonstrated lower peroxidase activity and polyphenol oxidation when compared with the samples treated with the commercial additive. These results confirm, at pilot scale and under industrial production conditions, the efficacy of the seaweed extract as a bio-based substitute for the synthetic coatings which are currently used to prevent browning in fresh-cut apples.

Keywords

Natural additive, Macroalgae, Peroxidase activity, Browning, Quality parameters, Industrial application.

Novelty Impact Statement

Fresh-cut fruits are subjected to processing operations leading to a decrease in nutritional and organoleptic properties. It is therefore necessary to adopt strategies to delay the degradative processes. In this study, the efficacy of a pilot-scale production and industrial application of a coating formulated with *Codium tomentosum* seaweed extract has been established for the first time. This seaweed extract possesses the potential to prevent browning development in fresh-cut apples under industrial operating conditions.

1. Introduction

The consumption of fresh-cut fruits is progressively increasing, and it accounts for a significant proportion of sales for horticultural processing companies (Putnik et al., 2017a). The cutting process inevitably triggers physiological responses and oxidation leads to visual changes (e.g. browning) occurring, which results in the fresh-cut products having a significantly lower shelf-life than the whole fruit (Khan et al., 2021). Most fresh-cut produces currently available have approximately a 7-day shelf-life, and there is a need to improve upon this figure without compromising on product quality attributes (Khan et al., 2021; Prakash et al., 2018). Extending the keeping quality of fresh-cut produce will contribute to market expansion, increasing producers' competitiveness and reduce food waste and losses. In the United States, about 45 – 55 % of food that is squandered annually are from horticulture crops, especially fruits and vegetables (Mitelut et al., 2021).

The application of edible coatings after cutting, provides a physical barrier against moisture and solute migration and lowers respiration rates (Saba & Sogvar, 2016). These edible coatings not only possess antimicrobial and antioxidant compounds which extend the keeping quality of the fruit, but also impart an attractive and glossy appearance to the fruit (Mitelut et al., 2021). Most coatings currently used contain components which are synthetic by nature, and there is considerable interest in replacing these coatings with natural alternatives (Chen & Xu, 2019). Although, only a few edible coatings are commercially available, especially for use in fresh-cut fruits, some coatings e.g. NatureSeal® and FOOD freshly® are reported to be used by the industry (Nicolau-Lapeña et al., 2022; Olivas & Barbosa-Cánovas, 2005). In the case of fresh-cut apples, substances like citric and ascorbic acids, calcium and thiol-containing compounds, and browning enzyme inhibitors, have been incorporated into coating formulations (Krasnova et al., 2017; Siroli et al., 2015). More recently, a number of other new coating formulations have emerged. Zha et al. (2022) observed a reduction in browning development in fresh-cut apples treated with riboflavin after 8 days of storage at 4 °C - an effect which was

related to the reduction in the activities of polyphenol oxidase and peroxidase, as well as the enhancement in phenolic content of the samples. Using a more complex formulation, Zhao et al. (2021) proposed a chitosan coating combined with *S*-nitrosoglutathione to decrease the oxidative stress in fresh-cut apples, and consequently inhibit browning of over 4 days of storage at 4 °C. Another source of efficacious components to achieve the same objectives could be seaweeds. Seaweeds are a natural source of bioactive compounds whose potential has been widely studied in food applications (Qin, 2018). The hydrocolloids extracted from seaweeds are widely used in food product formulations (Roohinejad et al., 2017). Alginate, agar, fucoidans, carrageenan and other hydrocolloids are examples of compounds extracted from brown and red seaweeds, which are widely used as texturing agents and stabilizers (Augusto et al., 2018; Roohinejad et al., 2017). Several studies have been reported on the use of antioxidants and antimicrobial compounds extracted from seaweeds, highlighting their benefits to human and animal health (Roohinejad et al., 2017). According to FAO, around 32 386.2 tonnes of seaweeds were produced for human consumption worldwide in 2018 (FAO, 2020). In addition to food product formulation, seaweed extracts possess a wide range of food applications. Augusto et al. (2016) investigated the use of seaweed-based coatings to preserve fresh-cut 'Fuji' apples. In this study, involving four distinct seaweed extracts - *Fucus spiralis, Bifurcaria bifurcata, Codium vermilara and Codium tomentosum* - conducted on a laboratory scale, the authors identified the extract of *C. tomentosum* as the most promising one to prevent browning in fresh-cut apples. The aforementioned extract significantly inhibited browning in fresh-cut apple slices even after 20 days of refrigerated storage under laboratory conditions (Augusto et al., 2016). In a more recent study, the authors investigated the efficacy of the *C. tomentosum* extract to inhibit superficial browning development in fresh-cut 'Rocha' pear slices (Augusto et al., 2022). In this study, after 15 days of storage at 4 °C, the samples treated with the seaweed extract exhibited lower colour changes and lower rates of superficial browning than a widely used synthetic commercial coating.

As these results were obtained on a laboratory scale, and commercial acceptance of this extract requires the validation of efficacy on a larger scale and under industrial conditions, the main focus of the present work is to validate the extract functionality on a pilot scale. A comparative analysis of the efficacy of this seaweed extract and an ascorbic acid based commercial formulation, which is currently used in industrial applications, has been carried out. The combined effect of modified atmosphere packaging on the shelf-life has also been investigated to assess whether the application of the extract can contribute towards a reduction in product loss. In summary, this research aims to provide evidence for validating the efficacy of the seaweed extract on a commercial scale.

2. Methods

2.1. Materials and Chemicals

Fuji apple was obtained from a local supplier in Torres Vedras, Portugal (Campotec S.A.) and stored at 4˚C before use. Dried milled seaweed *Codium tomentosum* having a particle size of 1.5 mm was purchased from ALGAplus (Ílhavo, Portugal). The ascorbic acid based commercial formulation, currently used in fresh-cut fruit production, was provided by Campotec S.A.

2.2. Seaweed extract preparation

A batch of seaweed extract was prepared from the dried milled seaweed purchased, by using a solid-liquid extractor (Pilotdist SL5®, Meckenheim, Germany) operating in a batch mode. A total of 1 980 g of dried seaweed, sieved through a mesh of 1 µm, was added to 30 L of a mixture of water and ethanol (75/25 v/v) taken in the extractor and contacted for 3 hours at 15 ˚C. The sieving process allowed robust solid-liquid contact and a clear extract was obtained at the end of procedure. This process is a scaled-up version of the extraction described by Augusto et al. (2018) and Augusto et al. (2016). After the contact time, 15 L of the liquid seaweed extract was collected and evaporated

at 35 ˚C (90 mbar) (Evaporator IKA, HB10+RV10, Germany) to remove most of the solvent. The residue was frozen at -80 ˚C, freeze-dried (Scanvac, Cool Safe™, Lyng, Denmark), and stored protected from light exposure at room temperature until further use.

2.3. Immersion coating of cut apples

Slicing and immersion of 'Fuji' apples in the seaweed extract was performed in a controlled temperature facility (2 ˚C) at Campotec S.A.. Two dip solutions were prepared: 1) a 5 % (w/v) aqueous solution of ascorbic acid based commercial formulation (currently employed in commercial products), and 2) a 0.5 % (w/v) aqueous solution of *Codium tomentosum* extract. Control samples of apple slices were obtained by simply dipping the slices in deionised water. Prior to slicing, apple fruits were disinfected with a solution containing 0.002 % (w/v) of sodium hypochlorite.

A total of 45 kg of 'Fuji' apples, with an average weight of 100 g, were automatically de-cored and sliced in a Turatti Splitter automatic slicer (Turatti, Italy), and 6.5 kg of slices were immediately placed on a conveyor belt running through 15 L of dip solution so that the slices were immersed for 2 minutes (Figure 1). The occluded dip solution was allowed to drain whilst the slices were still on the conveyor, following which the coated slices were transferred to the packaging system (Ishida, Kyoto, Japan and Ulma, Spain), where the slices were automatically divided into portions of 70.97 ± 10.72 g, packaged in plastic bags using a modified atmosphere (MAP) consisting of 1 – 8 % O_2, 12 – 22 % CO_2 and 70 – 87 % N_2 (for MAP samples) and air for ambient samples packaged only with atmospheric air followed by heat sealing. After packaging, the samples were transported under refrigerated conditions (5 ˚C) and protected from light exposure for 45 minutes from Campotec S.A in Torres Vedras (Portugal) to MARE- Polytechnique of Leiria in Peniche (Portugal), to simulate transportation between the producer and the consumer. A total of 534 packages of sliced apples, representing combinations of two dip solutions and two modified atmospheric conditions (89 packages per condition), were stored for 30 days at 4 ± 2 °C. The effects of treatment on fresh-cut apple quality were assessed

every 5th day after storage for 30 days by undertaking physicochemical analyses, enzymatic assays and microbiological analyses. Fresh-cut apple samples analysed immediately after cutting were used as a *gold standard* for comparing the treated and stored samples.

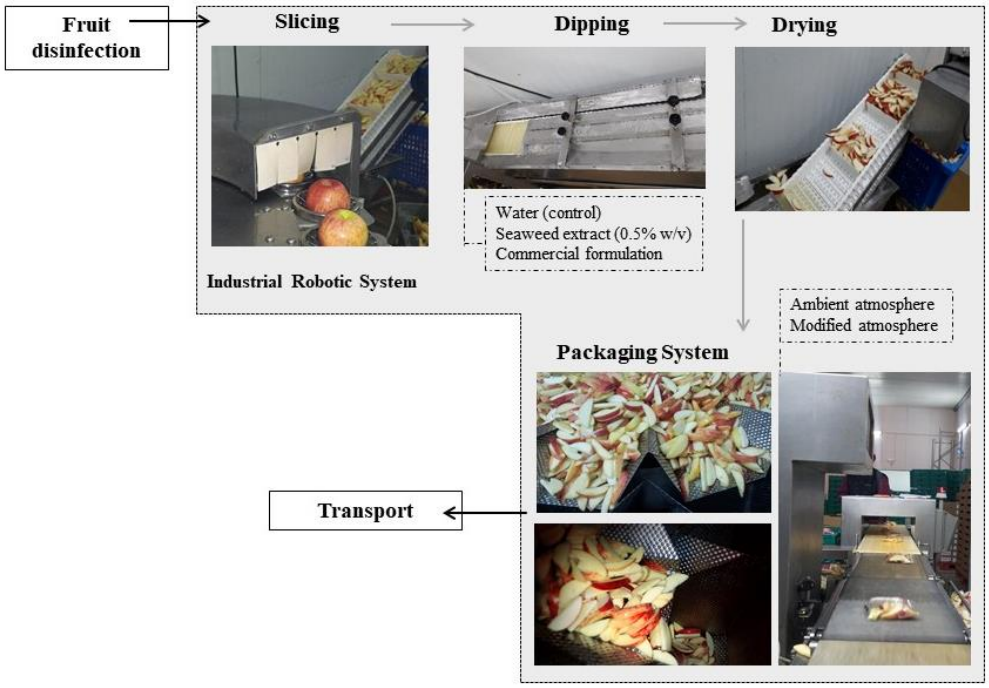

Figure 1. Schematic representation of coating process and packaging system.

2.4. Physicochemical analysis

The moisture content of apple slices was determined with an automatic moisture analyser (HB 43-S; Mettler Toledo, Giesen, Germany). A portable analyser (HP23-AW-A, Rotronic, Bassersdorf, Swiss) was used for water activity (a_w) measurements. The pH of apple slices was measured by the direct contact between the pH measuring probe and the sample surface (Inolab pH/ION, WTW, Germany). A digital refractometer (RFM340-M, Bellingham + Stanley, Xylem Analytics, Germany) was used for the determination of soluble solids content (SSC). For each of the determinations, three

separate measurements were performed (one per package) and the average difference between samples on day 30 and the *gold standard* was calculated.

A texture analyser TA.XT.plus (Stable Micro Systems, Surrey, England) was used to determine sample firmness as described in Augusto et al. (2016). Briefly, a 5 mm cylindrical probe was used to penetrate samples to a depth of 5 mm at a speed of 1.5 mms^{-1}. Firmness was defined as the maximum force required to perforate the apple slice and expressed in Newton (N). Fifteen measurements were taken for each condition (5 per package).

Colour parameters were analysed according to the CIELAB system as described in Augusto et al. (2016). A Konica Minolta portable colourimeter (CR 400, Japan) was used to measure the colour at three locations on each slice, one at the centre and two near the edges of the slices. The mean value for each slice was considered to determine the colour parameters. Results were expressed as intensity of lightness (L* parameter) and browning index (BI) (Augusto et al., 2016). The Eucladian distance of two points (ΔE*) was calculated between an individual sample and *golden standard* according to the equation described by Lante et al. (2016). Fifteen measurements were performed for each condition (5 per package). *Gold standard* samples were also assessed (n = 3) in terms of moisture, aw, SSC, pH, texture and colour.

2.5. Enzymatic assays

Polyphenol oxidase (PPO) and peroxidase (POD) activities were determined according to the procedure of Augusto et al. (2022). PPO and POD extractions were performed by homogenising frozen samples in 50 mM sodium phosphate buffer (pH 7.0) containing polyvinylpyrrolidone (PVP) (50 g L^{-1}), followed by a 30-min centrifugation step (12,000 g at 4 °C) for collecting the enzyme fraction. For both enzymes, the protocols were adapted for a reaction volume of 300 µL using a multi-well plate. For PPO determination, the

reaction was followed at 400 nm and catalysed by mixing the enzymatic fraction with a substrate mixture containing 20 mM catechol in 5 mM sodium phosphate buffer (pH 7). The determination of POD activity was undertaken by mixing the enzymatic fraction with a substrate mixture containing 1 % (v/v) guaiacol and 0.30 % (v/v) of hydrogen peroxide and prepared in a 0.05 M sodium phosphate buffer (pH 6.5). The reaction was followed at 470 nm during 10 min. The results were expressed as U mg^{-1} protein. Protein was quantified spectrophotometrically using the Bradford methodology (Bradford, 1976). Three different samples were analysed for each condition (1 per package).

For pectin methylesterase (PME) activity determination, the methodology was adapted from Augusto et al. (2022). The PME reaction was followed spectrophotometrically (Biotek, SynergyH1, USA) at 35 °C (610 nm, 4 min). To a volume of 50 µL of enzyme extract (pH 7.5), 15 µL of 0.01 % bromothymol blue (in 0.003 M sodium phosphate buffer, pH 7.5) and 235 µL of the substrate (5 g L^{-1} citrus pectin, pH 7.5) were added. Results were expressed as U mg^{-1} protein. Three different samples were analysed for each condition (1 per package).

2.6. Microbiological analysis

The mesophilic bacteria, Enterobacteriaceae, yeast and mould counts were determined by the procedure described in ISO 4833-1 (2013), ISO 21528-2 (2004) and ISO 21527-1 (2008), respectively. Samples solutions and dilutions were prepared in buffered peptone water according to ISO 6887-4 (2017). Mesophilic microorganisms were enumerated after 72-hour incubation at 30 °C in plate count agar. For Enterobacteriaceae enumeration, samples were incubated in violet red bile glucose agar for 24 hours at 37 °C. Yeast and moulds enumeration was performed after 7-days incubation at 25 °C in dichloran rose bengal chloramphenicol agar. Three packages for each condition were analysed for each sampling day.

2.7. Sensory evaluation

Two independent triangular tests were carried out according to the standard ISO 4120 (2004). First, three samples coated with commercial coating or seaweed extract solutions (see point 2.2) were presented to 21 untrained panellists. In the second test, the panellists were presented with untreated control samples and samples treated with seaweed extract. In both tests, the panellists had to identify the different samples. The sensory test was performed immediately after coating application (day 1) in a room complying with ISO 8589 (2007). Red lighting was used to avoid discrimination due to colour differences, and the sample presentation was randomized (Perez-Gago et al., 2006).

2.8. Statistical analysis

The results were statistically evaluated by one-way analysis of variance (ANOVA) with the Least Significant Difference (LSD) test for multiple comparisons of the means group. The evaluated variables were: coating solution, packaging and storage time. All data were checked for normality and homoscedasticity. The non-parametric test Kruskal-Wallis was used when the data did not meet variance or distributional assumptions. Differences were considered statistically significant at level 0.05 ($p < 0.05$). The software IBM SPSS Statistics 24 (IBM, New York, United States) was used for all calculations. Wherever suitable, results were expressed as mean ± standard deviation (n = 3).

3. Results and discussion

3.1. Physicochemical properties of fresh-cut apple during storage

Table 1 shows the results of moisture, water activity, pH and soluble solid content (SSC) variation (in %) of fresh-cut apple slices after 30 days of refrigerated storage when compared to the gold standard.

No differences were observed for moisture content between treatments for each packaging type ($p > 0.05$). The observed moisture increment (an average of 3 %) is possibly a consequence of the low temperature and high moisture content in the storage environment which may have resulted in water vapour transfer from the surroundings to the packages (Augusto et al., 2018). An increase in water activity was observed for all groups of samples. The lowest variation (approx. 50 %) was measured in control samples under modified atmosphere ($p < 0.05$) when compared with the commercial and seaweed extract coated samples packaged with and without modified atmosphere. The pH value of untreated and treated samples ranged between 3.6 and 4, and no statistical differences were observed between samples ($p > 0.05$), indicating quality maintenance of the fruits over storage in both types of packaging. The pH values noted were comparable to the study of Augusto et al. (2016).

293 **Table 1.** Variation (Δ) of moisture, pH, water activity and soluble solids content values
294 between packaged samples (ambient and modified atmosphere) at day 30 and gold
295 standard.
296

Packaging	Sample	Δ **Moisture (%)**	Δ **Water activity (%)**	Δ **pH (%)**	Δ **SSC (%)**
Ambient atmosphere	**Control**	2.44 ± 0.62^{A}	1.70 ± 0.22^{A}	-0.73 ± 2.90^{A}	-5.71 ± 4.17^{A}
	Commercial	3.72 ± 1.35^{A}	1.46 ± 0.21^{A}	1.19 ± 2.18^{A}	-14.15 ± 0.68^{B}
	Extract	3.82 ± 1.10^{A}	1.56 ± 0.18^{A}	-1.65 ± 0.88^{A}	-7.11 ± 1.12^{A}
Modified atmosphere	**Control**	2.11 ± 2.58^{A}	0.52 ± 0.28^{A}	1.74 ± 1.37^{A}	-17.09 ± 3.31^{A}
	Commercial	1.52 ± 2.71^{A}	1.60 ± 0.16^{B}	-2.01 ± 0.88^{A}	-10.73 ± 2.58^{B}
	Extract	3.02 ± 1.98^{A}	1.49 ± 0.16^{B}	-0.55 ± 4.41^{A}	-9.41 ± 3.73^{B}

297 Data are expressed as mean value ± standard deviation (n = 3). Values with the same packaging
298 with different superscripts (A-B) are significantly different (LSD test, $p < 0.05$).
299

300 The initial soluble solid content (SSC) of the gold standard samples and fresh-cut
301 samples was 14.07 ± 0.94 $g_{sucrose}$ 100 $g^{-1}_{product}$, which is within the same value range
302 stated in Lee et al. (2022). For all groups of samples, a decrease in SSC (Table 1) was
303 observed. The greatest decreases in SSC was observed in the case of samples treated
304 with the commercial solution and control, packaged with ambient and modified
305 atmospheres, respectively. This may be attributed to the possibility of microbial
306 metabolization of sugars which is dependent on the soluble solid content (Putnik et al.,
307 2017b). On the other hand, in the study developed by Augusto et al. (2016), an increase
308 in SSC values, in coated and uncoated fresh-cut apples after 20 days of storage, was
309 observed and attributed to moisture loss observed in samples.
310 Ripeness occurs in climacteric fruits during storage, and one of the main consequences
311 is firmness loss. This softening requires the use of techniques to prevent ripeness and
312 consequent textural quality decrease (Guerreiro et al., 2017). Since texture is related to
313 structural and mechanical food properties and an important parameter for consumer's

acceptance, the effect of treatment type, package and storage time on texture parameters were evaluated. After 30 days of storage, most sample groups had a firmness decrease of about 17 % ($p < 0.05$). However, samples treated with the seaweed extract and stored under modified atmosphere were the only samples to increase firmness by 26 %, which may evidence the advantage in the association between the seaweed extract coating and the use of modified atmosphere in samples storage. This is consistent with earlier research by Augusto et al. (2016) which showed that fresh-cut 'Fuji' apples coated with seaweed extract were firmer than water-treated control after 20 days of refrigerated storage. These observations establish the efficacy of the seaweed extract in maintaining textural attributes of fresh-cut apples even after scaling up the process to pilot scaling and under industrial conditions, in particular when associated with modified atmosphere packaging

The luminosity (L*), browning index (BI) and colour differences (ΔE*) of stored apple slices are shown in Table 2. In the CIELab system, L* defines luminosity on a scale that varies from black (0) to white (100) (Matos et al., 2021). In fresh-cut apples, higher values of L* are associated with the intensity of whiteness index and consequently lower oxidation in samples. A sharp decrease in L* values with storage time was observed ($p < 0.05$) for all treatments. However, on day 1, samples coated with the seaweed extract and packaged with ambient atmosphere showed about 44 % higher luminosity values (L*) ($p < 0.05$) than control and commercial samples. The same trend was observed in the samples packaged under modified atmosphere. The difference in luminosity observed between samples on day 1 can be explained by the rapid coating application and high efficacy of the seaweed extract during storage: browning is initiated on the surface of the fresh-cut apple during slicing (which induces enzymatic and non-enzymatic reactions leading to superficial darkening) with a consequent decrease in L* values (Shao et al., 2018). After 30 days of storage, for both types of packaging, no differences were observed between the commercial and seaweed extract treatments ($p > 0.05$), indicating similar darkening of tissues, which also suggests similar anti-browning

protection offered by both the commercial extract as well as the seaweed extract. When comparing both types of packaging, no statistical differences were observed for each set of treatments ($p > 0.05$).

Table 2. Colour parameters of Luminosity (L*), browning index (BI) and colour differences (ΔE*) of fresh-cut apples packaged with ambient and modified atmosphere at days 1 and 30 of storage at 4 ˚C, and *gold standard* samples.

Packaging type/ Sample		Storage time (days)/ **L***		Storage time (days)/ **BI**		Storage time (days)/ **ΔE***	
		1	30	1	30	1	30
Gold standard		77.75±6.01^{Aa}		42.56±4.95^{Aa}		n.a.	
Ambient atmosphere	**Control**	65.56±3.59^{B,1}	47.72±4.57^{B,2}	116.14±19.97^{B,1}	147.79±45.98^{B,2}	23.04±4.30^{A,1}	32.26±5.22^{A,2}
	Commercial	69.24±4.26^{B,1}	68.76±5.36^{C,1}	52.31±8.05^{C,1}	64.17±21.17^{C,2}	9.20±4.01^{B,1}	12.44±5.11^{B,2}
	Extract	85.03±2.48^{C,1}	62.83±7.32^{C,2}	40.12±8.91^{A,1}	91.49±27.15^{D,2}	19.73±1.54^{C,1}	18.37±7.29^{B,2}
Modified atmosphere	**Control**	70.56±4.22^{b,1}	58.52±9.99^{b,2}	92.06±17.62^{b,1}	129.74±28.93^{b,2}	17.12±4.46^{a,1}	26.03±6.24^{a,2}
	Commercial	69.33±3.71^{b,1}	64.88±6.60^{b,2}	70.60±10.07^{c,1}	69.32±22.61^{c,2}	12.36±3.01^{b,1}	14.27±7.02^{b,2}
	Extract	70.35±3.61^{b,1}	65.96±8.38^{b,2}	69.94±8.25^{c,1}	70.95±17.91^{c2}	11.88±2.57^{b,1}	13.64±7.74^{b,2}

Data were expressed as mean value ± standard deviation (n = 10). Results with different superscripts are significantly different in each day^{A,B,C} in ambient atmosphere and ^{a,b,c} in modified atmosphere, and between days$^{1 \text{ and } 2}$ (LSD test, $p < 0.05$). n.a.: not applicable.

The development of brown colour on the surface of fresh-cut apples is usually a manifestation of browning induced reactions due to the activity of polyphenol oxidase and peroxidase enzymes, making browning index an important parameter to be followed during the storage (Zha et al., 2022). On the first sampling day, the samples treated with

359 the seaweed extract and packaged under ambient atmosphere showed browning index
360 values similar to the gold standard samples ($p > 0.05$) (Table 2), while significantly higher
361 browning index values were measured in control and commercial samples ($p < 0.05$). In
362 modified atmosphere packaged samples, a higher browning index, in all coated samples,
363 was observed in comparison to the gold standard ($p < 0.05$), although a lower value than
364 in control samples ($p < 0.05$). In fresh-cut apple slices, the induction of browning is rapid
365 and almost instantaneous (Liu et al., 2021), which accounts for the observed differences
366 between the browning index values of the gold standard sample and other day 1 samples
367 - a process that seems inevitable even in the industrial scale process, despite close
368 monitoring and control of temperature. To avoid sample loss due to browning at an initial
369 stage, the temperature of the industrial facilities where the work was conducted was set
370 to 2 °C. All sets of samples stored for 30 days showed higher values of browning index
371 ($p < 0.05$), than gold standard samples and samples at day 1 – which is an expected
372 result, considering the number of storage days and the natural development of apple
373 browning (Fan et al., 2018). When individual treatments were compared and associated
374 with ambient atmosphere, samples treated with the commercial coating showed the
375 lowest values of browning index ($p < 0.05$), followed by the samples coated with the
376 seaweed extract. The observed lowering of browning of the commercial additive, even
377 without the use of modified atmosphere, is probably due to the presence of ascorbic
378 acid and calcium ascorbate, which are two commercial additives frequently used as anti-
379 browning agents in fresh-cut apples (Nicolau-Lapeña et al., 2022). Based on the findings
380 reported by Ramazzina et al. (2016), and more recently by Nicolau-Lapeña et al. (2022),
381 the efficacy of ascorbic acid based solutions to protect fresh-cut fruits against browning
382 is related to the regulation of oxidative stress by several mechanisms like reactive
383 oxygen species scavenging (ROS) and reduction of sugars. However, samples coated
384 with the seaweed extract showed similar values of browning index as the commercial
385 coating ($p > 0.05$), and considerably lower values than the control set ($p < 0.05$). To our
386 knowledge, this is the first time that the anti-browning functionality of the *C. tomentosum*

extract has been evaluated under industrial conditions, and therefore, the results suggest that it is possible to obtain a similar protection against browning as the commercial coating, even on an industrial scale, by combining the seaweed extract coating with modified atmosphere packaging. Despite the lack of statistical differences amongst the samples coated with the seaweed extract, the use of modified atmosphere enabled about 20 % lower browning index than samples packaged under ambient atmosphere.

Musacchi and Serra (2018) and Shao et al. (2018) state that the colour changes are an important and decisive factors determining consumers acceptance. The same holds true for the freshness of fresh-cut fruits (Belay et al., 2019), where higher variances in colour (ΔE*) is usually associated with product deterioration and lower coating efficacy. To ascertain the changes occurring in samples as a result of the different treatments and packaging conditions, the colour differences (ΔE*) were determined, and the outcome of this study is presented in Table 2. It is evident from Table 2 that samples treated with the seaweed extract and the commercial coating possessed similar colour after 30 days for both types of packaging, with lower values of ΔE* than control. All the results of colour parameters corroborate the earlier work of Augusto et al. (2016), and extend the effectiveness of the seaweed extract to a pilot-scale process when the cut slices are stored under ambient and modified atmosphere conditions.

3.2. Evaluation of browning related enzyme activities of fresh-cut apple slices during storage

Ripening associated processes originated by apple slicing are mainly promoted by polyphenol-associated browning, which is essentially triggered by the contact between the enzymes polyphenol oxidase (PPO) and peroxidase (POD) and their corresponding substrates, the phenolic compounds (Chen et al., 2021). These processes are accompanied by an increase in tissue respiration rate and an increase in enzymatic activity which in turn generate browning products like *o*-quinones (Oliveira et al., 2021).

Figure 2 shows the effect of coating solutions on PPO and POD activities during storage of the fresh-cut apple samples.

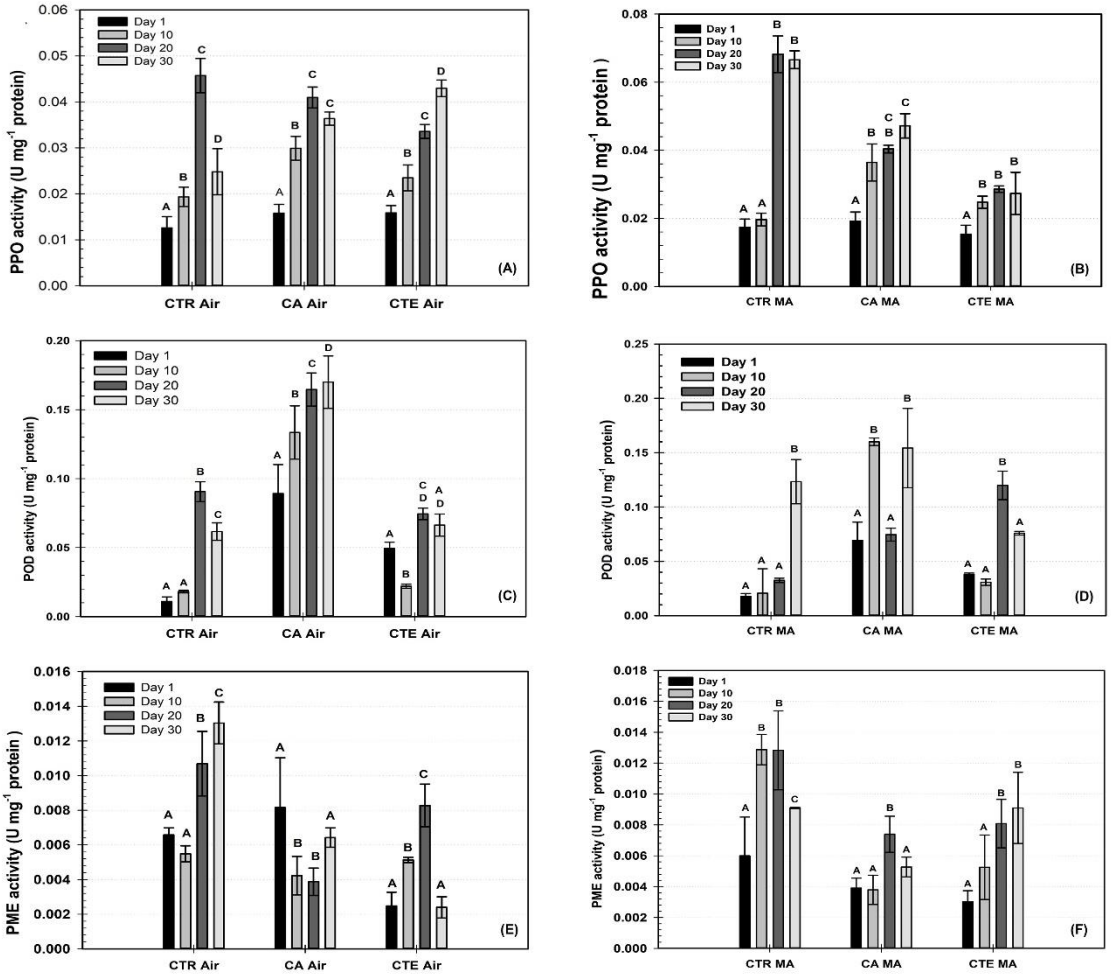

Figure 2. Polyphenol activity (PPO) (A-B), peroxidase activity (POD) (C-D) and pectin methylesterase (PME) (E-F) of fresh-cut apples packaged with ambient (left) or modified atmosphere (right). CRT- control, CA- commercial additive, CTE- *Codium tomentosum* extract. (Mean value ± standard deviation, n = 3). Values with different letters (A-D) in the same treatment are significantly different (LSD test, $p < 0.05$).

The enzyme PPO is considered the main browning-related enzyme responsible for the formation of *o*-quinones which are responsible for the formation of browning components in the surface of the fresh-cut apples (Chen et al., 2021; Oliveira et al., 2021).

Considering the results of PPO activity (Figure 2 A and B), all sample groups showed significantly higher PPO activity after 30 days storage ($p < 0.05$). At the end of the 30 days storage period, samples coated with the seaweed extract and packaged under ambient atmosphere gave higher PPO activity when compared to control and the commercial coating ($p > 0.05$), which may justify the higher values of browning observed in Table 2. In the case of samples stored under modified atmosphere, samples coated with the seaweed extract and stored for 30 days possessed the lowest PPO values (less 24 % to 58 % activity) when compared with the group of samples coated with the commercial solution ($p < 0.05$) (Figure 2B). From the observation of these results, it may be possible to hypothesise that in the presence of modified atmosphere the seaweed extract efficacy is enhanced, mainly when industrial conditions are applied, like those used in the present work. A result also evidenced by the differences in PPO activity observed between the two types of packaging in the seaweed extract group (air packaging = 0.0409 ± 0.003 U mg^{-1} protein; MAP packaging = 0.0273 ± 0.003 U mg^{-1} protein; $p < 0.05$). In previous studies performed at the laboratory scale with a duration of 20 days, the authors referred the reduction on the activity of PPO of fresh-cut apple slices coated with the seaweed extract during the storage period (Augusto et al., 2016). Also considered a key enzyme in browning processes through the oxidation of phenolic substrates (Chen et al., 2021; Oliveira et al., 2021), the activity of the enzyme peroxidase (POD) was assessed over the storage period as can be observed in Figures 2C and D. Observing the values of POD activity at days 0-30, samples coated with the seaweed extract had similar values of POD activity after 30 days when compared to day 0, demonstrating the efficacy of the seaweed extract at a pilot scale and for a longer duration than that reported by Augusto et al. (2016), which only evaluated the seaweed extract effect during 20 days of samples storage.

In the samples coated with the seaweed extract, the POD activity was 50 % lower than the samples coated with the commercial extract – which was the case for all sampling days ($p < 0.05$). The confirmation of these results for ambient as well as modified

atmosphere packaging reinforces the efficacy of the seaweed extract as an anti-browning edible coating which can be applied to fresh-cut apples processed under industrial conditions.

It may be noted that, in addition to oxidative enzymes, other enzymes like pectin methylesterase (PME) are also triggered into action soon after slicing. PME is involved in the apple ripening process by influencing cell wall degradation and causing loss of tissue firmness (Liu et al., 2021). It was therefore thought desirable to understand if the seaweed extract could influence PME activity just as it influences the activity of oxidative enzymes. Figures 2E and 2F show PME activity for all samples during the storage period. When slices are packaged under ambient atmosphere (Figure 2E) no specific trend is observed in PME activity over time. However, by comparing the results on days 1 and 30, a remarkable increase in activity of 49 % was observed in control samples ($p < 0.05$), while coated samples only showed a slight variation in PME activity ($p > 0.05$). It can also be highlighted that after 30 days, samples coated with seaweed extract had the lowest value of PME activity ($p < 0.05$) - 81 % and 63 % lower activity than the control and samples coated with the commercial extract, respectively. This may be due to the presence of polysaccharides in the seaweed extract which was described earlier by Augusto et al. (2018), which may be acting as a protection of the cell wall membrane against external damages as those induced by the cutting process.

Concerning samples packaged under modified packaging, it was possible to observe a consistently increasing trend in PME activity for all sample groups with storage duration (Figure 2F), although only control and seaweed extract sample groups showed a major increment in activity values ($p < 0.05$) of 34 % and 67 %, respectively. At the end of storage, and in contrast to ambient packaging results, the samples coated with the commercial additive presented significantly lower ($p < 0.05$) values of PME (0.002 U mg^{-1} protein). The possible presence of calcium in its formulation can explain this result since calcium is known to stabilize the integrity of the cell membrane and retard the action of PME (Aguayo et al., 2010).

3.3. Evaluation of microbiological counts in fresh-cut Fuji apple during storage

Fresh-cut apple is susceptible to microbiological degradation mainly due to cutting processes which increases the surface area and therefore the probability of contamination (Holban & Grumezescu, 2018). The European Commission regulation (EC No 2073/2005) requires evidencing the absence of *Salmonella* sp, *Escherichia coli*, and *Listeria monocytogenes*. The Portuguese government also recommends the control of mesophilic bacteria (less than 10^6 CFU g^{-1}), Enterobacteriaceae species (less than 10^4 CFU g^{-1}), and yeasts and moulds (less than 10^3- 10^5 CFU g^{-1}) (Santos et al., 2005). The results of the microbial analysis are shown in Table 3. After 15 days of storage, the mesophilic and Enterobacteriaceae bacteria counts in all samples were above the recommended threshold (10^6 CFU g^{-1}), so no further analysis was performed. However, the yeast and mould counts remained below the threshold values up to 25 days of storage. Regardless, the samples coated with the seaweed extract tend to present lower values of mesophilic, Enterobacteriaceae and yeasts and moulds counts in both types of packaging, demonstrating a possible antimicrobial effect of the seaweed extract, a result only recently reported by Augusto et al. (2022). In this work, the authors attributed the lower microbiological development in fresh-cut pear slices to the presence of C. tomentosum extract in the coating solution. A study conducted by Padhi and Tayung (2015) reported that *Codium decorticatum* seaweed contained several symbiotic microorganisms with antimicrobial activity which may also be present in the seaweed extract, thereby accounting for the observation. However, further studies are necessary to understand the role of seaweed extract as an antimicrobial component for preserving fresh-cut apple.

510 **Table 3.** Total viable counts, enumeration of *Enterobacteriaceae,* yeasts and moulds in
511 fresh-cut apples. Microbial counts above permitted threshold values are reported in bold
512 (Santos et al., 2005).
513

	Ambient packaging			Modified atmosphere packaging		
Storage time (days)	**Control**	**Commercial**	**Extract**	**Control**	**Commercial**	**Extract**
Mesophilic bacteria (Log CFU g^{-1})						
1	4.2	3.9	4.5	4.2	4.2	4.1
5	5.4	4.2	4.7	5.0	5.8	4.4
10	5.4	6.7	6.2	6.5	7.1	4.5
15	**7.1**	**7.3**	**6.8**	**7.1**	**7.6**	**6.5**
Enterobacteriaceae (Log CFU g^{-1})						
1	N.P.	2.8	N.P.	N.P.	N.P.	3.4
5	4.4	4.4	3.7	3.2	4.2	3.7
10	3.2	6.2	5.1	**4.0**	**4.8**	2.6
15	**6.2**	**7.1**	**5.4**	**4.4**	**7.3**	**4.2**
Yeasts and moulds (Log CFU g^{-1})						
1	N.P.	N.P.	N.P.	N.P.	N.P.	N.P.
5	2.8	N.P.	2.7	2.9	3.1	N.P.
10	3.0	4.5	4.2	3.1	4.4	2.7
15	3.6	3.4	3.3	3.4	3.7	2.7
20	4.3	4.4	4.3	6.5	5.4	2.7
25	4.3	5.3	4.6	3.8	5.2	4.4

514 N.P.- Not present.

515
516

517

518 **3.4. Sensory analysis of coated and uncoated fresh-cut Fuji apple**

519 The commercial application of this extract, as a new postharvest treatment, is viable only
520 if its application does not compromise the organoleptic profile. Therefore, two triangular
521 tests were performed, helping understand consumers preferences on the different
522 samples.

In the first test, panellists were instructed to identify the different sample between the seaweed extract and the commercial additive coated samples, statistical differences were identified ($p < 0.05$), indicating that there are organoleptic differences between commercial and seaweed extract coated samples. From the consumers comments, an "off-flavour" was present in the commercial samples, which was not reported in seaweed extract coated samples. The same "off-flavour" was also reported in fresh-cut apples treated with a calcium-ascorbate solution (Aguayo et al., 2010). In the second test, panellists were instructed to identify whether differences could be perceived between control and samples coated with the seaweed extract. No statistical differences were identified, supporting that extract application doesn't significantly alter apple organoleptic attributes.

4. Conclusions

The results of the present work contributed to the understanding of the pilot plant scale testing of a seaweed extract coating used to preserve fresh-cut apple slices, showing for the first time, the use of a natural coating applied under industrial conditions. A batch of 15 L of seaweed extract was produced using a pilot-scale solid-liquid extractor, and its functionality was assessed in ambient and modified atmosphere packaged samples. Different effects were found depending on the coating and packaging type as well as storage duration, depicting different scenarios after 30 days of storage:

Samples stored under modified atmosphere and coated with the seaweed extract

i) had similar textural attributes to fresh-cut apples on day 1.

ii) gave identical browning index and colour change values to those coated with the commercial additive.

iii) showed lower browning-related enzyme activities when compared with the commercial coated samples.

iv) showed delayed microbial growth.

v) did not influence the organoleptic quality as evidenced through sensory triangular tests.

Simultaneously, the observed reduction in the activities of the browning-related enzymes, and the delay in microbial growth in these samples, may be considered relevant factors for the scale-up validation of the seaweed extract. The results clearly show the benefits of seaweed extract coating, especially when associated with modified atmosphere packaging, thus establishing for the first time the efficacy of the seaweed extract under industrial conditions.

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Author Contributions

Ana Augusto: Conceptualization, Formal analysis, Methodology, Writing- Original Draft; **Andreia Miranda:** Formal analysis, Methodology; **Leonor Costa:** Formal analysis;

Joaquina Pinheiro: Methodology; **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:** Supervision, Conceptualization, Writing - Review & Editing, Funding acquisition.

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