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ß-Farnesene Exogenous Application as a Novel Damage Induction Model to Fast Explore the Effectiveness of Postharvest Strategies: The Case Study of the 'Rocha' Pear DOP

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Abstract: Since the prohibition of diphenylamine, replacement strategies have been needed for long-term disorder prevention, namely superficial scald (SC), in fruit. However, as this disorder only appears after months under cold storage, the assessment of effective strategies to prevent this disorder requires long periods. To tackle this challenge, we report in this paper a rapid and reliable system to induce symptoms, such as SC, based on storage under a β-farnesene-enriched atmosphere. Using this model, SC symptoms in 'Rocha' pear were induced after 15 d at 20 °C. As proof of concept, this model system allowed the study of the efficiency of antioxidant natural-based coatings on 'Rocha' pear quality maintenance. Pears treated with the coatings were submitted to 4 months of commercial storage under normal atmosphere conditions and the results were compared with those obtained using the induction model system. A PCA of chemical data allowed us to conclude that the model developed simulates the potential of certain strategies to prevent disorders.

Keywords: β-farnesene; damage induction model; postharvest quality; 'Rocha' pear quality

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1. Introduction

Given their perishable nature, pears are preserved using cold storage after harvest, which can be extended, in general, up to 10 months under a controlled atmosphere (CA) [1]. However, prolonged cold exposure can trigger postharvest physiological disorders, decreasing the shelf life and marketability of fresh pears. This quality deterioration is usually described as a combination of various necrotic injuries on the peel and/or flesh of pears, referred to as superficial scald (SC) and internal browning, respectively [2,3]. SC is one of the most common and problematic disorder in the postharvest field. The symptoms of this chilling-induced oxidative disease are generated during cold storage, but become intensified when the fruit are transferred to room temperature [4]. It has already been demonstrated that SC appearance in most pear cultivars is associated with the production of α-farnesene and their auto-oxidation into conjugated trienols [5,6]. This oxidative breakdown and redox imbalance lead to cellular disruption by conjugated trienols [7,8]. When this membrane damage occurs, cellular compartmentalization is disrupted allowing polyphenol oxidase (PPO) mediated browning [9]. It has also been proposed that SC is

a consequence of an imbalance between the fruit capacity to produce and/or regenerate antioxidants and hence scavenge oxidative species during cold storage [10,11].

Until 2013, the standard postharvest procedure in the postharvest industry to reduce the incidence of SC was the application of the antioxidant diphenylamine (DPA) combined with CA storage [12]. However, DPA use was forbidden, in 2011, due to the environmental and health hazardous effects already reported [13]. Since then, different approaches have been attempted to control this postharvest crop quality, such as CA storage, intermittent warming, and treatments with 1-methylcyclopropene (1-MCP) [3,14]. Nevertheless, the negative outcomes associated with these techniques, such as cost and sensorial quality constrains, demonstrated that these treatments are not as effective as DPA and not universally feasible per se, and also that their effect depends on the pear cultivar [15].

In recent years, the use of edible coatings has shown high potential to delay fruit quality deterioration during cold storage due to their potential in reducing respiration rates due to the formation of a gas barrier on the fruit surface [6,16–19]. In addition, such coatings can be loaded with food-compatible antioxidants, which can delay the occurrence of SC [3,6]. Such natural antioxidants can possibly scavenge oxidation processes by binding free radicals, protecting fruit from the oxidative process associated with superficial scald [7], and have been studied among several vegetable crops [20]. For instance, Sharma and Rao [21] reported that xanthan-gum-based edible coating loaded with cinnamic acid led to a reduction in the oxidative browning process on pears.

Effective research about new alternatives to prevent SC development has been hindered by the lack of an experimental system that can rapidly induce this disorder and allow several repetitions. Generally, as SC manifestations occur generally after 4 to 6 months in cold storage, the time necessary to achieve conclusions about new strategies success is frequently restrictive. To overcome this limitation, various attempts have been developed to artificially induce SC in a short period, focused on changing the air composition at room temperature, but usually without success [22]. Later, flushing with N_2 (anaerobiosis creation) after several days proved to provoke SC on apples [23,24]. Recently, Karagiannis et al. [4] found that a cold storage atmosphere enriched with ozone (O_3) induced scalding symptoms in 'Granny Smith' apples, but the technique failed regarding time, economic and environmental factors.

Since natural intrinsic α-farnesene oxidation is one of the reactions believed to explain the occurrence of SC symptoms, it is a hypothesis that the exogenous application of its isomer, β-farnesene, and its oxidation products, are also likely to induce similar disorder development. β-farnesene is one of the major isomers belonging to the farnesene family of acyclic sesquiterpenes found in the volatile emissions of a wide range of fruits and plants [25–27]. Additionally, its extraction and purification are less expensive than α-farnesene. In this context, the current study is designed to develop a novel approach to induce tissue damage based on the creation of a β-farnesene atmosphere through the natural volatilization of a pure β-farnesene solution. This model may be useful as a model for the quick exploration of the effectiveness of new treatments for superficial scald mitigation.

The aim of this work is to assess the reliability of β-farnesene enrichment in the storage atmosphere as a model system to study the SC physiological disorder in pears. The efficacy of the model was validated using pears treated with two natural extracts with different antioxidant potential: the methanolic leaf extract of *Arbutus unedo* L. tree, which demonstrated a high antioxidant activity and a subsequent potential to inhibit SC and quality preservation effect on 'Rocha' pear; and the methanolic apple byproduct extract from apple pomace with a weaker antioxidant activity [6,28]. To validate the results of 'Rocha' pear quality treated with these two natural-based extracts under the induction model, the treated pears were tested under common storage conditions during 4 months under normal atmosphere. Biochemical data supporting the hypothesis that β-farnesene-induced scald involves similar physiological and biochemical mechanisms to those which contribute to naturally occurring superficial scald are also presented.

2. Materials and Methods

2.1. Material

HPLC-grade methanol (≥99.9% purity) was supplied by Honeywell Riedel-de Haën AG, Seelze, Germany. ABTS (2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid, diammonium salt), food grade pectin from citrus peels, sodium carbonate, ascorbic acid, polyvinylpolypyrrolidone (PVPP), phenylmethanesulfonyl fluoride (PMSF), EDTA, catechol and guaiacol were purchased from Sigma-Aldrich, Missouri, EUA, and potassium persulfate and Folin–Ciocalteu from Merck, Damstadt, Germany. Glycerol was supplied by Fisher Chemical, New Hampshire, EUA. β-farnesene (99%) was kindly provided by Amyris, California, EUA.

2.2. Natural Extracts Screening and Selection

For validation of the β-farnesene atmosphere model system, two levels of potential antioxidant activity were defined (higher and lower antioxidant capacity compared to ascorbic acid). This selection, among different plants and byproducts, was performed in a previous work [28]. The natural extract with the highest antioxidant capacity was *A. unedo* L. tree leaf and the worse was the apple byproduct. For further analysis, concentrations of 9.5 g L^{-1} and 16 g L^{-1} were used for *A. unedo* L. leaf and the apple byproduct, respectively.

2.3. Fruit Material and Extract Material

Pear fruits (*Pyrus communis* L. 'Rocha') were harvested at optimal maturation stage from a 30-year-old commercial orchard in Cadaval (39°25′ N; 8°54′ W; 120 m), Portugal, in August 2018. The pears were transported immediately after harvest to a commercial packinghouse and hand-sorted to select undamaged fruit. The fruit were then stored at 4 °C and 95% RH until processing. The pear fruits were kindly provided by COOPVAL (Bombarral, Portgual).

Fresh *A. unedo* L. leaf was provided by Medronhalva, LDA, and stored at—20 °C. Before extraction, the leaf was freeze dried and ground into a fine powder (IKA A10 analytical grinder). The apple byproduct was provided by INDUMAP-Fruit's Industrialization, Portugal, stored at—80 °C upon arrival, and used directly for extraction after thawed. The extraction procedure was performed according to [6]. Each plant material (1:20 m/v) was consecutively extracted three times with methanol for 1 h under constant stirring at 25 °C with the renewal of the solvent between extractions. The suspensions were then vacuum filtered, MeOH was removed in a rotary evaporator (Büchi rotavapor R-114) and the extracts were freeze dried to obtain the final extract.

2.4. Preparation of the Pectin-Based Coating and Fruit Coating

Pectin coating was prepared according to the method reported by Oms-Oliu, Soliva-Fortuny, and Martín-Belloso [29] with some modifications. The coating was prepared by dissolving pectin (3% w/v) in distilled water and heating at 60 °C while stirring until the solution became clear. After cooling down to room temperature, the chosen natural extracts were dissolved in the coating at the concentration previously optimized (9.5 g L^{-1} and 16 g L^{-1} were used for *A. unedo* L. leaf and the apple byproduct, respectively). The pears were dipped into the coating solution for 2 min and allowed to drain for 1 min and to dry at room temperature for 2 h. Uncoated pears were used as experimental controls, and pears coated without the incorporation of natural-based extracts (i.e., only pectin) were used as procedural controls.

2.5. β-Farnesene-Atmosphere-Based Model

2.5.1. Model System Optimization

To induce the occurrence of tissue damage, samples of 6 pears were equally distributed in polypropylene boxes with 5 L (24.5 cm × 13 cm × 16 cm) capacity and exposed to different volumes of pure β-farnesene (optimization results in Section 3.2) under a controlled temperature (25 °C). After the selection of the β-farnesene volume (150 mL), the protective

potential of the antioxidant coatings under the β-farnesene atmosphere was evaluated. The pears were removed from cold storage and dipped in the different postharvest treatment coatings and allowed to dry before being exposed to pure β-farnesene. The pears were randomly divided into two lots with 24 fruit each, 1 lot was packed in 4 plastic boxes, hermetically covered and used as control (i.e., without β-farnesene), and the other lot was packed with the optimized volume of β-farnesene. The 24 pears of each lot were divided into 4 experimental conditions (experimental control; procedural control; *A. unedo* L. leaf coating (AL.C); and apple byproduct coating). This experimental design was performed in triplicate. Quality measurements were performed immediately after coating dryness (0 d) and then at 15 d of storage under each condition and atmosphere. Visual inspection for damage development was assessed daily.

2.5.2. Model System Validation Using a Pilot Scale Storage of Pears

Further reproducibility of the results, obtained with the β-farnesene-atmosphere induction model system, was achieved with a pilot scale storage of pears across a 4 month commercial storing period at Cooperativa Agrícola dos Fruticultores do Cadaval (COOPVAL). Pears from this time had already 4 months of cold storage since harvest. The pears were randomly divided into 3 groups of 50 fruit each. The pears of the first group were treated with AL.C, whereas the second group was treated with the coating without incorporation of natural-based extracts (procedural control) and the third group remained untreated (experimental control). The fruits from each group were cold stored (0 °C, 95% RH), a normal 'Rocha' pear storage strategy at the agricultural cooperative. Randomly, 10 pears from the 3 different treatments were sampled and analyzed for quality and superficial scald development at 5 different storage times (0, 1, 2, 3 and 4 months). Following cold storage, the pears were allowed to ripen for 7 days at room temperature (18–20 °C), hence simulating the marketing period. In parallel for each treatment and each sampling, three replicates of two pears each were isolated and subsequently frozen in liquid nitrogen and stored at—80 °C for biochemical analysis.

2.6. Determination of Pears Physicochemical Parameters

Fruit quality was evaluated in terms of firmness, surface color, total soluble solids, pH and scald incidence. Fruit surface color was measured with a CR-400 colorimeter (Konica Minolta, Osaka, Japan) using the D65 illuminant and the CIE (Commission Internationale de l'Eclairage) parameters (L*, a*, b*). Hue was calculated as hue angle (h° = arctan (b*/a*)). Photos were also taken daily to report the pears' overall appearance. Two measurements were performed on opposite sides of the widest part of each fruit. Firmness (expressed as N) was measured using a texturometer (T.A. XT plus Texture Analyser, Stable Micro Systems, Cardiff, UK) fitted with a 5 mm diameter probe. Force calibration was performed with a 5 kg load cell and height calibration was performed for each pear. The soluble solids content (SSC) was measured in the fruit juice by a digital refractometer PR1ATAGO CoLTD (Japan). For pH measurements, a sensION^{TM} PH31, HACH (Spain) was used. SC incidence was recorded visually in each sampling day and calculated as a percentage of the total number of fruit affected per condition per month [30].

2.7. Antioxidant Capacity and Phenolic Content (Extraction and Quantification)

The extraction of antioxidant compounds from pears was performed according to Salta et al. [31] with some modifications. Thawed pear samples (5 g) were previously homogenized in a Moulinex stirrer and then extracted with 50 mL methanol (3×), while stirring for 15 min. The mixture was filtered with two layers of Miracloth and the liquid extract evaporated under vacuum at 50 °C using a rotary evaporator (Büchi Rotavapor R-114). The concentrated extract was volumetrically adjusted to 10 mL for further analysis with methanol. All the extractions were performed in triplicate with two biological replicates and reading in duplicate.

The total antioxidant capacity was determined by the 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) free radical scavenging assay, as described elsewhere [32]. After the addition of 1.0 mL ABTS solution to 10 µL of the sample, the mixture absorbance reading was performed after 6 min at 743 nm. With the calibration curve, previously prepared with ascorbic acid as a standard, the result was expressed as ascorbic acid equivalent concentration (AEAC) per g of pear on a fresh weight basis.

2.8. Determination of the PPO Enzyme Activity of Pears

PPO was extracted and analyzed as described by Galeazzi and Sgarbieri [33]. Pear tissue (10 g) was homogenized for 2 min in an ice bath with 10 mL of 200 mmol L^{-1} sodium phosphate buffer pH 6.5, 0.25% (*v*/*v*) Triton X-100 and 2% (*w*/*v*) of PVPP, using an Ultra-Turrax. The obtained solution was centrifuged at 4000× *g* for 20 min at 4 °C. The supernatant was filtered through two layers of Miracloth and used as enzyme extract. For the activity determination, the enzyme extract was mixed with the reaction buffer, which consisted of a 200 mmol L^{-1} catechol and 200 mmol L^{-1} of sodium phosphate buffer pH 6.5. The catechol oxidation rate was evaluated at 420 nm for 3 min at room temperature.

All the extractions were performed in triplicate with two biological replicates and readings in duplicate. Enzymatic activities were expressed as the change in unit of activity (U) per milligram of enzyme responsible for a change in 1 absorbance unit per minute. The total protein content in the enzyme extracts was determined using the Pierce Coomassie Plus Protein Assay Kit (Pierce ThermoScientific Inc., Rockford, IL, USA), based on the method of Bradford, following the manufacturer's instructions. Bovine serum albumin was used as a standard.

2.9. Statistical Analysis

Differences between extracts antioxidant activities were tested by the one-way analysis of variance (ANOVA). One-way analysis of variance was applied to determine differences between treatments in both atmospheres (i.e., in the presence and absence of β-farnesene). Additionally, a two-way analysis of variance was performed to assess differences on the pilot scale assay using the treatment and month of storage as independent factors. Fisher's least significant difference (LSD) was conducted for mean comparisons. Differences with a probability value of <0.05 were considered significant and all data were reported as mean ± SD. ANOVA analyses were conducted using STATISTIC software (StatSoft v.8, US). A principal component analysis (PCA) of the chemical parameters was performed using Statgraphics Centurion XVII.

3. Results and Discussion

3.1. Selection of Natural-Based Extracts of Antioxidant Action

The processing of plant foods results in byproduct production, which can be considered rich sources of bioactive compounds, notably compounds with antioxidant properties. Among the bioactive compounds present in plants, phenolic compounds are widely appreciated for their strong antioxidant potential, due to their double bounds and ability to delocalize electrons removing free radicals [31,34,35]. Therefore, in a previous study [28], an initial screening of natural-based extracts with antioxidant activity to be used in edible coatings to prevent pear SC was carried out. The results allowed the selection of the extracts with the highest and lowest antioxidant potential compared to ascorbic acid. In particular, the samples selected were *A. unedo* L. leaf extract (highest antioxidant capacity) and apple byproduct extract (lowest antioxidant capacity). Indeed, in a previous work, we demonstrated the high variety of phenolic compounds found in *A. unedo* L. leaf, which could justify their considerable antioxidant activity [28,35,36]. Additionally, in a previous study [6], AL.C demonstrated to preserve 'Rocha' pear quality during cold-storage.

Although apple byproducts extracts have been reported to be rich in phenolic acids [28], the obtained results show that they display a poor antioxidant activity [37]. Hence, because of their different behavior regarding antioxidant capacity, and therefore their different

potential in reducing pear scald, these extracts were used as a case study to test the β-farnesene-atmosphere model system developed in this study.

3.2. β-Farnesene-Atmohphere Model System Optimization and Evaluation

Since α-farnesene oxidation is one of the main biochemical reactions responsible for the appearance of scald symptoms [5], it was our objective to create a simple and fast model system to induce SC damage through the application of the α-farnesene isomer, β-farnesene, under a controlled temperature (25 °C). Various attempts to find the optimal β-farnesene volume, to induce SC in the manner of the naturally occurring SC, in the 5 L headspace boxes were performed (data not shown). The optimization of β-farnesene volume was carried out because we observed that much higher volumes than 150 mL led to the rapid injury of the fruit skin. As it was used in the case of the pure substance in a closed system and considering the vapor pressure 1.333 Pa at 25 °C [38], the more energetic β-farnesene molecules diffused, i.e., evaporated, from the liquid to the gaseous phase until reaching saturation. This equilibrium allowed the contact of β-farnesene with pears responsible for the appearance of physiological damage. A volume of 150 mL, at the center of each 5 L box and without stirring, with six fruits placed at represented in Figure 1 for 15 d, were determined as the conditions to induce physiological damage in pears, i.e., 30 mL β-farnesene per L of container capacity and 25 mL β-farnesene per pear. The effect of the antioxidant edible coatings in the quality preservation of pear under the β-farnesene-atmosphere model system was then evaluated (Figure 2 and Table 1).

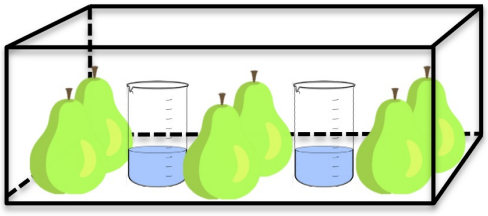

Figure 1. Experimental design of β-farnesene damage induction model system.

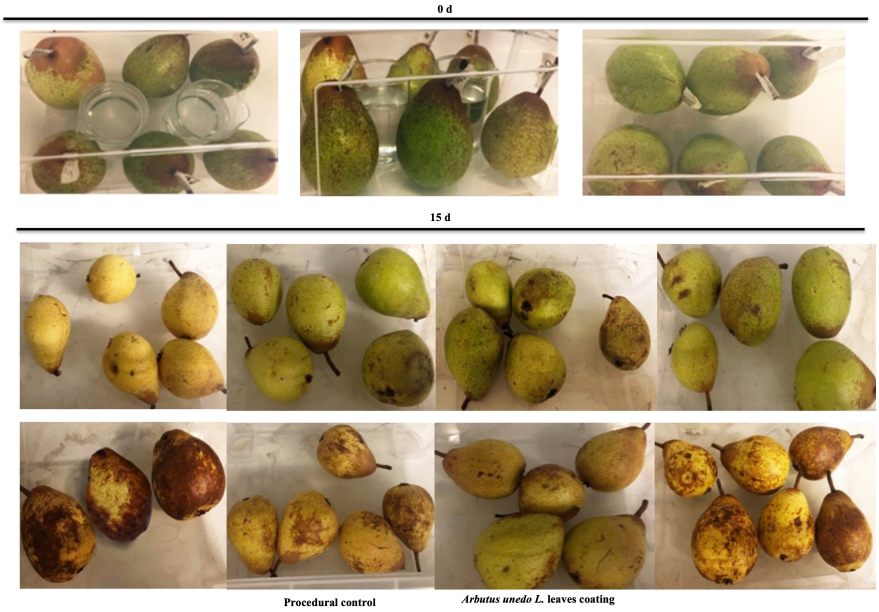

Figure 2. 'Rocha' pear fruit at the initial (0 d) and final (15 d) stage of β-farnesene damage induction model system.

Table 1. Lightness, hue angle, firmness, soluble solid content, pH, scald index, AEAC and PPO activity of 'Rocha' pear at 0 and 15 d storage under the atmosphere without (−) and with (+) β-farnesene. Means ± standard deviation of six determinations followed by the same lower-case letter indicates no differences between conditions at each time point, and by the same upper-case letter indicate no differences between storage months at each condition at $p < 0.05$ (n = 6).

Quality Parameters			0 d	15 d
Lightness (L*)	−	Experimental control	64.58 ± 0.95 a	71.72 ± 2.27 A
		Procedural control	63.21 ± 2.45 a	67.38 ± 3.33 B
		AL.C	66.13 ± 5.20 a	68.87 ± 1.36 B
	+	Experimental control	60.73 ± 2.91 a	79.47 ± 3.36 C
		Procedural control	65.64 ± 1.97 a	72.46 ± 3.26 A
		AL.C	64.66 ± 5.57 a	67.87 ± 3.36 B
Hue angle (h°)	−	Experimental control	106.74 ± 3.07 a	87.20 ± 0.80 A
		Procedural control	103.91 ± 3.30 ab	93.81 ± 1.50 C
		AL.C	103.83 ± 3.42 ab	97.76 ± 4.39 D
	+	Experimental control	104.97 ± 4.66 ab	82.88 ± 1.45 E
		Procedural control	105.35 ± 2.47 ab	88.03 ± 0.88 A
		AL.C	102.21 ± 1.80 b	103.82 ± 4.40 B
Firmness (N)	−	Experimental control	33.26 ± 1.67 a	6.28 ± 0.29 A
		Procedural control	34.24 ± 1.77 a	8.04 ± 0.39 B
		AL.C	35.90 ± 5.30 a	11.58 ± 2.26 C
	+	Experimental control	36.40 ± 3.24 a	6.38 ± 0.78 A
		Procedural control	33.85 ± 1.67 a	7.65 ± 0.39 AB
		AL.C	36.59 ± 3.04 a	9.81 ± 1.47 D
SSC (%)	−	Experimental control	12.13 ± 1.26 a	14.15 ± 0.59 AC
		Procedural control	12.42 ± 0.43 a	13.62 ± 0.45 AB
		AL.C	13.85 ± 0.55 b	13.38 ± 0.37 B
	+	Experimental control	13.42 ± 0.45 b	14.27 ± 0.60 C
		Procedural control	12.45 ± 0.31 a	13.40 ± 0.32 B
		AL.C	12.52 ± 0.38 a	13.02 ± 0.74 B
pH	−	Experimental control	5.66 ± 0.17 ac	4.79 ± 0.05 A
		Procedural control	5.67 ± 0.22 ac	5.48 ± 0.16 B
		AL.C	5.88 ± 0.17 b	5.14 ± 0.24 C
	+	Experimental control	5.96 ± 0.15 b	4.79 ± 0.17 A
		Procedural control	5.82 ± 0.13 ab	5.17 ± 0.09 C
		AL.C	5.60 ± 0.10 c	4.72 ± 0.10 A
SC incidence (%)	−	Experimental control	0	0
		Procedural control	0	0
		AL.C	0	0
	+	Experimental control	0	100
		Procedural control	0	83.30
		AL.C	0	16.67
AEAC (g kg^{-1})	−	Experimental control	0.64 ± 0.08 a	0.56 ± 0.02 A
		Procedural control	0.61 ± 0.06 a	0.19 ± 0.04 B
		AL.C	0.67 ± 0.09 a	0.60 ± 0.01 C
	+	Experimental control	0.60 ± 0.10 a	0.69 ± 0.01 D
		Procedural control	0.62 ± 0.04 a	0.23 ± 0.03 B
		AL.C	0.66 ± 0.03 a	0.62 ± 0.04 C
PPO (U.mgprotein^{-1})	−	Experimental control	2.95 ± 0.66 a	3.11 ± 0.43 A
		Procedural control	2.72 ± 0.34 ac	2.75 ± 0.38 AC
		AL.C	3.52 ± 0.48 b	2.28 ± 0.34 B
	+	Experimental control	2.64 ± 0.45 ac	2.95 ± 0.43 AC
		Procedural control	2.41 ± 0.11 c	2.59 ± 0.23 BC
		AL.C	2.67 ± 0.42 ac	1.67 ± 0.19 D

3.2.1. Visual Pear SC-Like Symptom Evaluation

The results reported in Figure 2 and Table 1 show that it is possible to produce typical pear SC-like symptoms within 15 d by exposing the fruit to the atmosphere containing β-farnesene. It is clear from Figure 2, by comparison with the controls in the presence and absence of β-farnesene, that under this conditions damage can be observed after 15 d of storage on β-farnesene-treated pears. It is clear from Figure 2 that the β-farnesene atmosphere accelerated the senescence of the fruit since pears exposed to the β-farnesene atmosphere showed a more damaged surface. In contrast, coated pears showed a greener and healthier surface compared to the control pears, which suggests the protection given by the coatings. α-farnesene biosynthesis appears to promote ethylene production [39]. Similarly, β-farnesene could induce the ethylene biosynthesis responsible for faster senescence.

The application of edible coatings to pears can create a barrier to gas diffusion inside the fruit, leading to a reduction in the senescence process, which can explain the greener surface of coated pears not exposed to β-farnesene [16]. However, the application of an external stress to the fruit resulted in scald-affected and more senescent fruits. Pears with AL.C substantially reduced such visible injury (Figure 2). Additionally, and as expected by its lower antioxidant activity, pears treated with the apple byproduct coating showed higher levels of damage. These results are in agreement with the antioxidant activity of these two extracts, which may be responsible for the protective effect observed. While *A. unedo* L. leaf extract presented a higher antioxidant potential and, therefore, was more effective to reduce tissue injury and senescence, the apple byproduct with a much lower antioxidant activity did not prevent pear damage. Uncoated pears were the ones showing more browning injuries.

The extract with higher antioxidant content, *A. unedo* L. leaf extract, reduced peel damage. Additionally, the antioxidants of this extract coating allowed the fruit to be more protected. Thus, the physicochemical data present in Table 1 supports the visual evaluation performed regarding the effect of AL.C on the protection of fruit from damage, since the apple byproduct was not visually so effective.

3.2.2. Physicochemical Properties of Pears

Color

Initially, immediately after the application of the coating on pears, L* was measured (Table 1). In fact, at application time, the color of pears with AL.C was not different from the controls showing that the optimized concentration of *A. unedo* L. tree leaf extract in the coating had a seamless effect on the pears' color (Figure 2). In fact, the development of edible coatings with optical clarity is desirable, assuring a seamless effect when applied to the fruit [40]. After 15 d of storage, there was an increase in L* and a decrease in h° values (Table 1) in all conditions and storage conditions, which is characteristic of 'Rocha' pear ripening [12,41]. Nevertheless, surface color was affected by the presence of β-farnesene. Higher ΔL* and higher Δh° values indicate faster ripening and damage development [3] and were observed in pears under β-farnesene conditions, particularly in the experimental control, compared to pears under no β-farnesene atmosphere. Additionally, it is clear from the differences between the coated pears and the experimental control under no β-farnesene atmosphere that coatings slowed the ripening process (lower Δh° compared to the control). In pears, the decrease in L* values is a result of chlorophyll degradation and the accumulation of carotenoids. The treatment with edible coatings inhibited the chlorophyll breakdown of various horticultural products, including pears [42]. Pears with the AL.C demonstrated (in both storage conditions) the maintenance of a lower ΔL* and Δh° values, as proved by differences with the control, confirming its protective role. Oms-Oliu et al. [29] also observed that the incorporation of antioxidants into coatings effectively maintained the h° values of freshly cut pears.

Firmness

Firmness is one of the main fresh pear quality parameters. Its decrease is associated with transpiration and respiration processes during storage and cell wall degradation, which promote moisture and turgor loss, leading to fruit softening [40,43]. After 15 d of storage, a rapid softening trend was evident in all samples, although the rate of decrease was different. Despite not statistically different from the uncoated pear, pears coated only with pectin showed a lower firmness loss, which can be due to the coating effect itself (i.e., limited gas diffusion (low O_2), which limits the degradation of the pectins present in the peel). Pears with AL.C better maintained firmness compared to uncoated and pectin-coated pears (Table 1), which indicates the potential use of AL.C on delaying fruit senescence. The higher firmness preservation observed can be attributed to the limited gas diffusion (low O_2) after the application of the edible coating plus the enrichment with antioxidants, which limit the activities of oxidizing enzymes [44]. These enzymes are responsible for turning insoluble protopectins into the more soluble pectic acid and pectin, which, consequently, leads to less membrane integrity degradation [45,46]. Under the β-farnesene atmosphere, a higher firmness loss was observed, probably due to the external stress promoted by β-farnesene, but the same behavior was observed for all conditions.

Soluble Solids Content (SSC) and pH

It is well known that sugars are an important parameter defining fruit maturity and quality. Therefore, it is crucial that the postharvest treatment minimizes cellular processes to maintain the total soluble solids for a prolonged time. After the application of the coating, SSC values generally increased after 15 d of storage, as part of the natural ripening. It was expected that SSC values found in pears stored in the presence of β-farnesene were higher, because of the metabolism induction pattern observed with the other quality parameters, but no differences between the two storage conditions (with and without β-farnesene) were observed. On the other hand, differences were found between the coated and uncoated pears, stored in both conditions, especially between the pears treated with AL.C and the control. This lower accumulation of sugars in the treated fruit can be explained by the effect of the coating in reducing the respiration and metabolic activities associated with the hydrolysis of starch into sugar [3,17]. A decrease in the pH was observed, regardless of the storage conditions and atmosphere. An increase in pH values was expected in highly respiring fruit, such as in control conditions, particularly under the β-farnesene atmosphere since organic acids, such as malic and citric acids, are the primary substrates for respiration [47]. Inversely, in coated pears, the expected decrease in pH was observed since the coating delayed the ripening process and therefore the respiration avoiding the faster degradation of organic acids. The same results were obtained by Sharma and Rao [21], where xanthan-gum coatings were applied to freshly cut pears.

SC Index

At the initial sampling date, none of the conditions exhibited scald symptomatic pears. SC-like damage appeared after 15 d of storage only on pears under the β-farnesene atmosphere treatment. The presence of this α-farnesene isomer affected 100% of the control fruit, 83.30% of pears from the procedural control, and 16.67% of pears treated with AL.C. The analysis of the results suggests that it seems that β-farnesene volatilization effectively promoted stress and injuries in pear skins, leading to the appearance of SC-like signs. However, some protection was detected when pears were treated with AL.C. It is known that edible coatings created an atmosphere inside the fruit similar to the modified atmosphere, i.e., restriction O_2 entering, thus slowing down ethylene production and oxidative metabolism [3]. Additionally, as SC can be regulated by α-farnesene oxidation, the treatment with coatings with higher antioxidant capacity can explain the protection offered by AL.C. Thus, it appears that the bioactive compounds present in AL.C may be responsible for this protective effect, since the apple byproduct coating did not exert the same results (Figure 2). In fact, antioxidant systems might inhibit the occurrence of

oxidation injuries by neutralizing scald-related oxidation reactions [48,49]. Sharma and Rao [21] reported that xanthan-gum-based edible coatings incorporated with cinnamic acid lead to a reduction in the oxidative browning process. Given all this, it is possible to hypothesize that AL.C was able to protect pears from β-farnesene oxidation damages.

3.2.3. Antioxidant Activity of Pears

A higher antioxidant activity was observed in AL.C treated pears, which was maintained during storage. These results are supported by Correa-Betanzo et al. [50], who stated that the application of edible coatings helped to preserve the antioxidant activity of berry cactus fruit. The edible coating by itself (i.e., only pectin) led to a decrease in the antioxidant capacity of the pear as observed on Table 1. However, it is notable that the presence of the antioxidants from A.L. enhanced the antioxidant capacity of the fruit. These results are in agreement with Oms-Oliu et al. [29], which also demonstrated that the use of polysaccharide-based edible coatings by themselves did not contribute to the enhancement of the antioxidant capacity of freshly cut pears. Lindo-García et al. [30] presented that, although having initial higher antioxidant potential, a higher disorder incidence in controls was observed, demonstrating that the initial intrinsic antioxidant potential of the fruit was not directly related to the development of SC. The total antioxidant activity of pears varied between the AL.C treatment and the control.

3.2.4. Oxidative Enzyme Activity of Pears

In this experiment, the PPO activity of pears under the β-farnesene atmosphere showed coherence with the color changes observed, since oxidation via this enzyme is believed to be one of the major causes of skin browning [51]. In this regard, compared to the control, the treatment with AL.C suppressed the PPO activity, which corroborated the lower scald-like percentage observed. Conversely, the other conditions showed an increase in the enzyme activity, which explains the higher L* and lower h° values obtained. Additionally, pears under the β-farnesene atmosphere demonstrated higher increments of PPO activity corroborating the hypothesis of injury development. The natural antioxidants present in AL.C can potentially counteract oxidation-linked damages associated with PPO activity through its inhibition [21,52]. Additionally, the maintenance of higher membrane integrity in pears treated with AL.C explains the lower activity of PPO in pears treated with AL.C since this enzyme is localized in vacuoles. Thus, the lower cell membrane degradation promoted by these coatings contributes to reducing PPO activity, exhibiting less SC-like symptoms.

3.3. β-Farnesene-Atmosphere Model System Validation in Storage Conditions

Normally, in commercial storage, SC appears after 4–6 months of cold storage [5]. Therefore, a simulated commercial long-term storage trial was executed across 4 months to conclude about the reproducibility of the results obtained with the 15 d β-farnesene induction model system. Since, in the induction model experiment, AL.C demonstrated a potential protection function against damage development, the results focused on SC development and quality parameters in the coated pears with AL.C and controls (Figure 3).

3.3.1. Physicochemical Properties of Pears

Color

At the end of 4 month storage, AL.C preserved a higher chlorophyll pigment than both controls, which resulted in fewer variations of L* and h° values (Figure 3A,B). As stated before, these lower variations on color parameters could show the reduction in senescence and superficial scald incidence [53], which corroborates the results obtained previously. This limitation of color degradation is in accordance with a recent study on 'Bartlett' pears, which showed the positive effect of chitosan coating in reducing senescence [54].

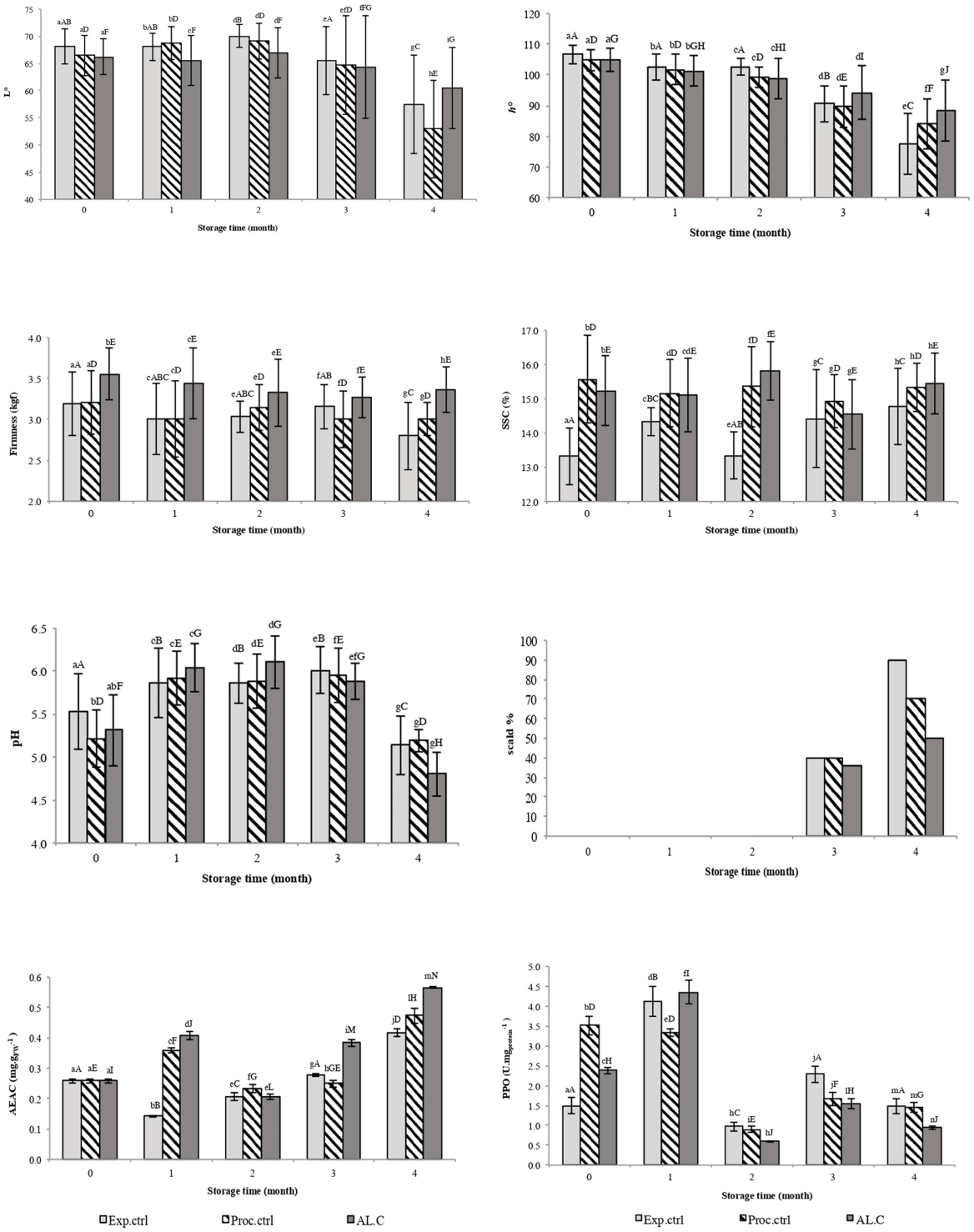

Figure 3. Color parameters (L* (**A**) and hue (**B**)), firmness (**C**), soluble solid content (SSC (**D**)), pH (**E**), scald index (**F**), AEAC (**G**) and PPO (**H**) activity of 'Rocha' pear at 0, 1, 2, 3 and 4 months of commercial storage. Exp. ctrl: experimental control; Proc. ctrl: procedural control; AL.C: *Arbutus Unedo* leaf coating. Error bars represent the standard deviations of the means (n = 10 for A, B, C, D, E, F; n = 6 for G and H). Same lower-case letter indicates no differences between conditions at each time point, and the same upper-case letter indicates no differences between storage months at each condition at $p < 0.05$.

Firmness

A retention of firmness on pears with AL.C was observed, and firmness loss was higher in uncoated pears (Figure 3C). It suggests that AL.C treatment slowed down the ripening process probably due to the oxygen barrier effect and to the indirect inhibition of the catalytic activity of carbohydrate-degrading enzymes. Therefore, once again, it demonstrates the reduction in the ripening and quality loss of pears [12,55].

SSC and PH

In the case of SSC, it is known that organic acids are degraded during ripening, while the sugar content increases [55]. As shown in Figure 3D, this increase was observed only in uncoated pears. Coated pears did not change the SSC content during storage. This lower variation of sugars in treated fruit might be to the effect of coating, which reduced respiration and the metabolic activity of pears, therefore less sugar was consumed [43]. The data regarding the effect of pectin coating incorporated with antioxidants on pH are presented in Figure 3E. As shown, there is an increment until the 3rd month and then a decrease, but without differences between the coated and uncoated pears. This decrease at the end of storage was also noticed on coated pears under the β-farnesene atmosphere and could be explained by the coating influence on respiration activity, which thereby delays the degradation of organic acids, such as the malic and citric ones [47].

Scald Index

The percentage of the SC surface was recorded as the total number of affected fruits in each condition. SC evaluation in uncoated pears indicates that, at the 3rd month, SC symptoms started to appear, and a remarkable increase is achieved at the end of storage, with uncoated pears reaching 90% of scald. Scald is lower in coated pears especially in pears with AL.C. This data are consistent with the findings obtained using the β-farnesene induction model system developed in this work. Given that scald is known to be the result of an oxidative process [5], antioxidants may play a decisive role in SC prevention. This corroborates that the higher antioxidant activity observed in pears with AL.C (Figure 3F) can give some protection against superficial scald development.

3.3.2. Antioxidant Activity of Pears

The data presented in Figure 3G show the changes in the antioxidant activity of coated and uncoated pears. In general, antioxidant activity increased until the end of storage. It is known that, in apples, the increase in antioxidant activity during storage is due to the biosynthesis of phenolic compounds [56]. However, higher antioxidant activity was detected in pears with AL.C, whereas a lower antioxidant activity was noticed in control fruit. These results validated the effects obtained previously under the induction model system atmosphere. The application of this postharvest technique helped to preserve the antioxidant activity better than control fruit. Likewise, the addition of antioxidants into the coating could explain the higher antioxidant activity observed.

3.3.3. Oxidative Enzyme Activity of Pears

The activity of polyphenol oxidase (PPO) enzyme was measured in uncoated and coated pears during storage. In this experiment, it was not possible to set a clear tendency about PPO activity over time. However, it is noticeable that, until the end of storage, there is a decline in PPO activity especially in pears with AL.C (Figure 3H). According to Ghasemnezhad et al. [57], the lower activity of the PPO enzyme can be interpreted as the inhibition of enzymatic browning and, therefore, less superficial scald development. The same conclusions were obtained by Kou et al. [58]. Additionally, the higher membrane integrity and greener surface observed in pears with AL.C explain the lower PPO activity measured. Overall, these findings relate to the lower SC development and validate the β-farnesene model as adequate for the quick exploration of the effectiveness of antioxidant coatings against superficial scald development in the 'Rocha' pear.

3.4. Principal Component Analysis of the Experiment

The principal component analysis (PCA) was performed to summarize and understand the relation between the results obtained under the induction model system with β-farnesene and commercial storage. As expressed before, the principal aim of this work was to create a simple and fast method to enhance the appearance of SC and to achieve conclusions regarding an effective treatment against SC in a short time frame. To prove the reliability of the model system, the same selected antioxidant coatings and controls were tested under a 4 month commercial storage. Figure 4A shows that the first and second principal components described 79% of the variability (53.32 and 25.68%, respectively). Figure 4B shows that the first and second principal components described 60.353% of the variability (37.368 and 22.986%, respectively). Both principal component analyses clearly separated the different conditions, which were mainly dependent on the presence of a higher antioxidant content, i.e., AL.C coating is separated from the other conditions. These PCA results corroborate the conclusions obtained in both assays. It is also clear that pears with AL.C are negatively related to PPO activity and L* values and positively related with firmness, which explains the observed capacity in reducing SC incidence in both assays (Table 1 and Figure 3). Hence, the PCA results demonstrate the reliability of the model system developed in this study, as proved by the similarity of the biochemical data obtained in both assays. Additionally, AL.C could delay ripening and reduce the SC of coated pears. Although previous results suggested that edible coatings enriched with natural antioxidants provide a defense against oxidative reactions and SC [43,52], not all antioxidant extracts demonstrated positive results, so the AL.C composition demonstrates a high potential for its application in the future as a solution to inhibit SC.

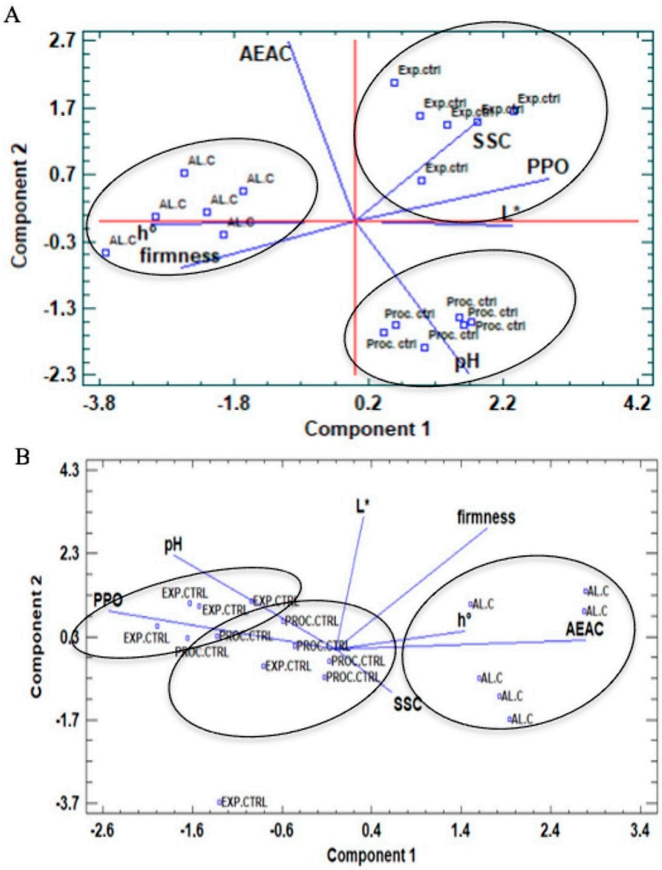

Figure 4. (**A**) PCA analysis biplot for the quality parameters of the β-farnesene atmosphere trial at the end of storage. (**B**) PCA analysis biplot for the quality parameters of the commercial trial at the end of storage. Exp. ctrl: experimental control; Proc. ctrl: procedural control; AL.C: *Arbutus Unedo* leaf coating.

4. Conclusions

This work evaluated an innovative damage induction model system to study SC prevention in postharvest pears through the exposure of fruit to a β-farnesene-enriched atmosphere. The conditions used were found to induce similar SC symptoms in 15 d. This innovative SC induction model represents a no-equipment, simple, rapid, reliable and inexpensive method to test the efficacy of treatments or postharvest strategies to prevent SC and, eventually, other postharvest disorders. The application of the natural extracts to 'Rocha' pears as in this case study permitted the demonstration of the model consistency along with the 4 month commercial storage at low temperatures, which opens the possibility of the application of this model induction to other fruit crops. With this model system and as expected, the protection offered by AL.C was corroborated, which enhanced the quality of pears and reduce injury under the β-farnesene atmosphere, and the same observations were obtained in the commercial trial. In this paper, it was demonstrated for the first time that the generation of a β-farnesene atmosphere can constitute a convenient injury induction model system to fast-track the effectiveness of new treatments and their doses to be applied in the postharvest sector, particularly in the fruit sector.

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