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Research Article

Treatment With Kiwi Peel Extract Delays Browning in Ready-to-Eat Lettuce

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Browning reactions caused by oxidative stress occurring during postharvest procedures significantly compromise the quality of ready-to-eat (RTE) vegetables, negatively affecting their market value. Polyphenol oxidase (PPO) and peroxidase (POD) are two major enzymes involved in this phenomenon, as they oxidize phenolic compounds to quinones, which in turn polymerize to brown pigments. Recently, there has been an increasing interest in developing antibrowning treatments using food by-products. Herein, the efficiency of a kiwi peel extract in reducing enzymatic browning of minimally processed lettuce (*Lactuca sativa*) has been investigated. PPO and POD activities showed an opposite spatial distribution within the leaf, with a higher POD activity in the midvein (MV) and in the inner lamina tissues, and a prevalence of PPO activity in the mesophyll. Considering that MV lignified tissues are those mainly affected by browning, the temporal trend of POD activity over a 20-day storage period at 4°C was investigated. Data showed that treatment with a kiwi peel extract hinders both the increasing trend of POD activity and browning development compared to control leaves. These results could be potentially useful for the industry as they confirm that natural extracts, such as kiwi peel extract, can be valuable for extending the shelf-life of RTE products.

1. Introduction

In industrialized countries, the demand for ready-to-eat (RTE) vegetables, including green leafy salads, is constantly growing. The commercial success of these products is due to their qualitative and technical characteristics, as they offer the opportunity of eating fresh, safe, healthy, and nutrient-rich products without taking time for preparation [1, 2]. In this context, organoleptic properties and shelf-life of RTE products are key elements in influencing consumer choice. However, some of the processes to which fresh-cut vegetables are subjected before packaging, such as cutting, peeling, and washing, as well as the use of some sanitizers, can affect both organoleptic properties and microbiological safety, reducing their overall quality [3–6]. Among these processes, mechanical damage resulting from cutting vegetables represents one of the main limits influencing the shelf-life of RTE lettuce, as the cutting of lettuce leaves causes wound-induced physiological and biochemical reactions that shorten storage life of the minimally processed product [7]. Among these, tissue browning is a typical event that affects fresh-cut lettuce. Browning is considered to result from an increase in enzymatic activities associated with the loss of cell integrity and compartmentalization that is provoked principally by cutting. Indeed, mechanical damage increases the activity of several enzymes involved in metabolic pathways leading to the production of phenolic compounds [8, 9], whose subsequent enzymatic oxidation to quinones, followed by spontaneous nonenzymatic polymerization, results in the accumulation of brown pigments

responsible for tissue darkening. Moreover, mechanical wounding facilitates the interaction between phenolic compounds and oxidative enzymes by compromising cellular compartmentalization, thus allowing the reactions to occur [10]. It has been shown that the lettuce tissue with the highest susceptibility to enzymatic browning is the "white" tissue located around the midvein (MV), which is the area that darkens most following minimal processing and further storage of RTE lettuce [11, 12]. Therefore, developing strategies to counteract fresh-cut lettuce browning has become a crucial issue to improve the quality of these products and extend their shelf-life.

The three key enzymes involved in phenol-dependent browning are phenylalanine ammonium lyase (PAL, EC 4.3.1.5), polyphenol oxidase (PPO, EC 1.10.3.1), and peroxidase (POD, EC 1.11.1.7). The first step in phenol metabolism is the PAL-catalyzed conversion, within the shikimic acid pathway, of the amino acid L-phenylalanine to cinnamic acid, a precursor of many phenylpropanoid compounds. The browning process has been demonstrated to be caused by the oxidizing action of enzymes such as PPO and POD on the phenolic substrates generated by the PAL-dependent pathway [13]. Although browning phenomena are generally assumed to be a direct effect of PPO activity on polyphenols, as PPO is the key enzyme involved in the melanogenesis pathway [14], there is no reported evidence that this process applies to all plant species. Regarding this, it has been reported that although POD is mainly involved in lignification processes, it can also form melanin pigments [15, 16] and its activity has been linked to browning in many plant species including lettuce [17, 18]. Indeed, POD can use hydrogen peroxide (H_2O_2) as a catalyst for the oxidation of phenolic compounds, including mono- and di-phenols [13]. In addition, it has been reported that POD enhances PPO-mediated browning reactions in a sort of coupled-browning [19]. In this regard, it has been hypothesized that PPO-mediated generation of quinones may lead to the accumulation of H_2O_2, which could prompt further browning by POD-mediated oxidation of polyphenols [20]. So, in addition to sharing common substrates, POD and PPO can also cooperate in dark pigment accumulation, with PPO providing H_2O_2 for POD-mediated browning [10, 20]. Both enzymes are present in lettuce [21–23] and their activities can be induced by the wound [18, 24] made during postharvest processing, leading to a significantly diminished quality of RTE product.

Over the years, various strategies have been adopted to keep enzymatic browning of fresh-cut vegetables under control [25]. Currently, the most commonly used preservation techniques include both physical and chemical treatments, such as cooling, modified atmosphere packaging, and treatments with antioxidants, useful to reduce the speed of biochemical reactions and the activity of the oxidizing enzymes PPO and POD [25, 26]. In particular, among antioxidant compounds, it is well known that ascorbic acid has a potent effect in preventing enzyme-catalyzed oxidations due to its reducing activity [27–29]. Indeed, the positive effect of a postharvest treatment with ascorbic acid as an antibrowning agent has been reported in different plant species including fruit plants (e.g., loquat fruit [30], apple [31], nectarine peaches [32]) and fresh-cut salads [19, 33]. Considering this, the use of plant extracts containing high levels of antioxidant compounds, such as ascorbic acid, to treat postharvest vegetables could have great potential in reducing processing-induced browning.

In this context, the use of natural compounds, such as food-derived bioactive molecules, for the extension of RTE vegetable shelf-life, can offer important advantages compared to other treatments, especially in terms of safety and sustainability, so that they could be more widely accepted by consumers [34]. Furthermore, given the development of the food industry and the consequent increase in the amount of waste, especially derived from fruit and vegetable by-products, it could be useful to promote the circular economy by recycling as much waste products as possible. For this reason, the attention has been recently focused on the use of natural antioxidants obtained mainly from waste by-products of the agri-food chain [35, 36]. Fruits used to produce beverages generate a huge quantity of waste products, such as peels, seeds, and pulp, which can contain a great number of bioactive compounds [37]. Furthermore, recent studies have shown that the peels of some fruits, such as grapefruit, pomegranate, and kiwi [34], have a high content of ascorbic acid, phenolic compounds, and flavonoids, thus maintaining a strong antioxidant power. In this context, many studies have recently focused their attention on the use of plant-based antioxidants as inhibitors of enzymatic browning. Akabari et al. recently analyzed the effectiveness of fruit flesh juices with high antioxidant activity which were obtained from pomegranate, kiwi, and grapes, on the storage of pear slices at 4°C. Results therein reported showed that the kiwi juice was the most effective in preserving the pear slices, in which a greater firmness of tissues and simultaneously a lower POD activity was found [38]. These results demonstrate that the antioxidant treatment can be obtained not only from the fruit flesh, but also from their by-products, such as peels. Indeed, recent evidence showed that peel, which is a main waste product of many industrial preparations, contains as much antioxidant compound content as the rest of the fruit tissues [34, 39]. Moreover, it has been shown that, compared to other green salad species, lettuce is one of the most susceptible to browning phenomena, presumably due to its lower content of ascorbic acid compared to the higher content of other green salads (i.e., rocket salad) [13]. In this context, the aim of this work was therefore to evaluate the impact of wounding and of the subsequent treatment with a kiwi peel extract on POD activity and browning of minimally processed lettuce (*Lactuca sativa* var. Longifolia) during a storage period of 20 days.

2. Materials and Methods

2.1. Plant Material and Chemicals. Lettuce heads (*Lactuca sativa* var. Longifolia) and mature kiwi fruits (*Actinidia deliciosa* var. Hayward) were purchased from a local retailer on the same day of the delivery by the agricultural supplier, considered as time 0 (T0). Lettuce heads utilized for all experiments were composed of nine whorls total, each

composed of four leaves. 4-Aminoantipyrine (AAP), sodium 3,5-dichloro-2-hydroxybenzenesulfonate (DCHBS), catechol, Coomassie Brilliant blue, and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Experimental Design for the Evaluation of Enzymatic Activity. To determine the spatial distribution of POD and PPO enzymatic activities in different leaf areas, the leaves were cut as follows: (i) longitudinally into two halves, with only one half including the MV; (ii) longitudinally into two halves, each one including the same amount of MV; (iii) transversally into a basal and an apical part; and (iv) in central and marginal leaf areas, obtaining the central leaf area, including the MV with the immediately adjacent tissue, and the external area, including marginal tissues and the lamina.

To determine POD and PPO enzymatic activities in the whole lettuce head, all leaves of each whorl (nine whorls total, each composed by four leaves), from the outermost (first whorl) to the innermost (heart), were sampled and separately analyzed at T0.

To determine the variation of POD activity during storage at 4°C, three leaves of a lettuce head were collected at T0, from the first, fourth, sixth, and seventh whorls. One leaf from each whorl was analyzed at T0, the other two leaves from the same whorl were stored in food containers at 4°C and analyzed after 15 days (T15) and after 20 days (T20), respectively. All leaves used for this part of the experiment were sliced transversally into five equal parts each approx. 3 cm (Supporting Figure S1a).

The determination of the variation of POD activity during storage at 4°C after treatment was performed using an aqueous extract of kiwi peel. For the obtainment of kiwi extract, kiwis were purchased from a local retailer and selected at the ripe stage and were peeled. The peels were blended using a Waring commercial laboratory blender for approximately 5 min after the addiction of distilled water (1: 2 w/v) while keeping the extract on ice in a glass beaker. The raw extract was used immediately for the treatment of the lettuce leaves. For each experimental replicate, two lettuce heads were used, one for control and the other one for treatment. For each head of lettuce, three leaves from both the fourth and the seventh whorls were sampled. The leaves (cut into five transversal sections) were immersed in the kiwi extract for 30 min (or in distilled water as a control) and then washed with distilled water and air-dried before being stored in commercially sourced clamshell containers at 4°C. The analyses were performed immediately after processing (T0) and after 15 (T15) and 20 (T20) days on the three sampled leaves independently.

2.3. Preparation of Enzymatic Fraction. For the evaluation of POD activity, fresh lettuce leaves were ground in a mortar at 4°C using 2 mL of 0.1 M sodium phosphate buffer (pH 7) per g of fresh weight. The homogenate was centrifuged at 12,000 g for 10 min at 4°C. The supernatant was used for the determination of POD activity.

For the evaluation of PPO activity, fresh lettuce leaves were ground in a mortar at 4°C using 5 mL of 0.1 M acetic acid–sodium acetate buffer (pH 5.5) per g of fresh weight. The homogenate was centrifuged at 10,000 · g for 30 min at 4°C. The supernatant was used for the determination of PPO activity.

2.4. Enzymatic Activity and Protein Determination. POD enzymatic activity was measured using a spectrophotometer by following the formation of a pink adduct ($\varepsilon_{515nm} = 2.6 \times 10^4\ M^{-1} \cdot cm^{-1}$) as a result of the H_2O_2-dependent oxidation of 4-AAP and the subsequent condensation of oxidized 4-AAP with DCHBS [40]. The standard reaction mixture for determining POD activity contained 400 μL of the supernatant, 100 μL of 1 mM AAP, 100 μL of 10 mM DCHBS, 390 μL of 0.1 M sodium phosphate buffer (pH 7), and 10 μL of 0.2 M H_2O_2 as the substrate. The volume of the final assay was 1 mL. The values of absorbance at 515 nm were measured (using the Beckman Coulter DU 730 spectrophotometer). No POD activity could be measured in the absence of H_2O_2 in the assay mixture. POD activity was expressed as $U \cdot g^{-1}$ fresh weight (U; 1 unit of enzyme activity is the amount of enzyme that catalyzes the oxidation of 1 μmol substrate per min).

PPO enzymatic activity was determined following previously described methods [41], with slight modifications. The standard reaction mixture for determining PPO activity contained 800 μL of the supernatant and 200 μL of 0.05 M catechol, as the substrate. The volume of the final assay was 1 mL. The values of absorbance at 420 nm were measured (using the Beckman Coulter DU 730 spectrophotometer). No PPO activity could be measured in the absence of catechol in the assay mixture. PPO activity was expressed as $U \cdot g^{-1}$ fresh weight (U; 1 unit of enzyme activity was defined as the amount of enzyme that produced 1 mmol of quinone per minute. A conversion factor of $U = 2717 \cdot \Delta$ optical density was used for the calculations) [42].

Protein content was estimated using the Bradford method with bovine serum albumin as a standard [43].

2.5. Browning Quantification. To quantify the browning phenomenon in both untreated leaves and leaves treated with kiwi peel extract during storage at 4°C, images of salad slices were captured after 20 days (T20). ImageJ 1.44 software was used to convert.jpg images in RGB-separated channel images. As the color features change during storage at 4°C, the frequency of the extracted color parameters (RGB) also changes for each treated and untreated leaf over time. These three features (R, G, and B values) are suitable for determining the dark pigment formation on the cut surface of salad slices. Each one of the R, G, or B values has a value from 0 to 255, and total RGB value is considered as the sum of these three parameters (R + G + B = 765). Considering that R, G, and B values associated with black color are 0, 0, 0, and the same values related to white are 255, 255, and 255, the browning associated with dark pigment accumulation is correlated with low RGB values. For each condition, three sliced leaves were photographed and

analyzed using ImageJ 1.44 software. RGB value measurements were carried out by drawing a region of interest (ROI) that includes the MV area of the transversal sections of the sliced leaf (Supporting Figures S1b and S2).

2.6. Statistical Analysis. To evaluate POD and PPO enzymatic activities in different leaf areas, three different lettuce heads were analyzed as independent experiments. For each experiment, three leaves were collected and analyzed from the lettuce head using the mean of the three values ($n = 3$). To evaluate POD and PPO enzymatic activities in the whole lettuce head (nine whorls total, each composed of four leaves), each leaf of each head-whorl was analyzed separately from three different lettuce heads and again the mean of the four values was used in the analysis ($n = 3$). To evaluate POD activity and to quantify the browning phenomenon during storage at 4°C (with or without treatment), three leaves of similar size taken from the fourth and the seventh whorls of three different lettuce heads were analyzed for each time-point ($n = 3$). For the determination of the total protein content, the same extract used for the evaluation of the enzymatic activity was used.

Statistical analyses were carried out with GraphPad Prism performing the one-way ANOVA followed by the Šidák multiple comparison test. Statistical significance of differences was determined with $p > 0.05$ as nonsignificant (ns); *, **, ***, and ****; and $p \leq 0.05$, 0.01, 0.001, and 0.0001, respectively.

3. Results and Discussion

3.1. POD and PPO Enzymatic Activities Are Differently Distributed in Lettuce Leaf Tissues. To evaluate the possible correlation between the POD and PPO enzymatic activities and the development of browning in different leaf zones, their spatial distribution was analyzed by separating the different leaf areas (Figure 1). Data show that no statistically relevant differences of POD and PPO enzymatic activity levels were found between the basal and the apical part of the leaf, nor between the left and right parts resulting from a longitudinal cut along the MV. On the contrary, statistically relevant differences were observed in the PPO and POD enzymatic activity levels between left and right parts resulting from a longitudinal cut including or not the MV, as well as between marginal and central zones of the leaf. Specifically, the two enzymes analyzed showed an opposite spatial distribution within the leaf, with a higher level of POD activity in the MV compared to the mesophyll (Figure 1(a)), and a prevalence of PPO activity in the mesophyll, compared to the MV (Figure 1(b)). This observation is supported by studies that have shown that plastid-resident PPO is abundant in chloroplasts of photosynthetic cells, especially in mesophyll of young tissues [44]. Secretory POD is localized in mature lignified tissues, where its activity is involved in cell wall stiffening and lignification events during development and defense responses [45]. Data herein reported are strongly correlated with this evidence, reporting a high POD enzymatic activity in the MV and in the inner lamina tissues, which are the principally lignified tissues of the leaf, as well as a high PPO enzymatic activity in the mesophyll, which is the most plastid-rich tissue complex (Figure 1).

3.2. POD Enzymatic Activity Is Higher in Outermost Lettuce Whorls. Spatial distribution analyses of POD and PPO enzymatic activities were carried out in the whole lettuce head (Figure 2). Each leaf of each whorl, from the outermost (first whorl) to the innermost (heart), was sampled and analyzed at T0. Data were analyzed both on fresh weight basis and in relation to total protein content to exclude nonspecific effects due to a variation of total protein content. The distribution of POD enzymatic activity, expressed both on a fresh weight basis (Figure 2(a)) and on total protein content (Figure 2(b)), between leaves derived from different whorls at T0 showed a decreasing trend starting from the external whorl toward the internal one, depending on developmental stages and lignification levels. No statistically relevant differences were found among the three outermost whorls (first–third whorls) in POD enzymatic activity levels, which however decreased starting from the fourth whorl, reaching basal levels in the three innermost whorls (seventh whorl-heart; Figures 2(a) and 2(b)). PPO enzymatic activity, expressed both on a fresh weight basis (Figure 2(c)) and on total protein content (Figure 2(d)), showed an opposite trend, increasing from the external older leaves of the first whorl toward the internal younger leaves of the sixth whorl. These data are consistent with previously reported evidence, according to which most of the PPO present in plants is located in plastids, such as chloroplasts of photosynthetic cells and leucoplasts of storage cells [44]. It has been also reported that the PPO abundance varies in both a time- and space-dependent manner within the same plant, showing higher PPO levels in young tissues and lower levels in mature and senescent tissues [46]. Moreover, the precise localization of POD and differential distribution of its oxidative substrates provide mechanisms to control spatio-temporal deposition of lignin during development [47]. This is in agreement with the higher levels of POD enzymatic activity found here in the lignified tissues of the mature lettuce leaves, seen as the oldest leaves of the outermost whorls. The increase in POD activity correlated with leaf age, and in particular the increment in the older leaves, was also previously reported for other plant species, such as tobacco [48] and maize [49]. Data herein reported demonstrate for the first time a direct correlation between PPO and POD activity levels and the developmental stage of leaves relatively to the whorls of a whole lettuce head. Data demonstrate that both POD and PPO vary widely along the whorls, starting from those composed of the youngest leaves to the most mature ones, highlighting an opposite trend for the two enzymatic activities that vary in a space- and time-dependent manner.

Given the lower PPO activity as well as the prevalence of POD activity in the MV and in the inner lamina tissues (the so-called "white tissues"), together with the evidence that in lettuce the lignified tissues are the ones mainly affected by

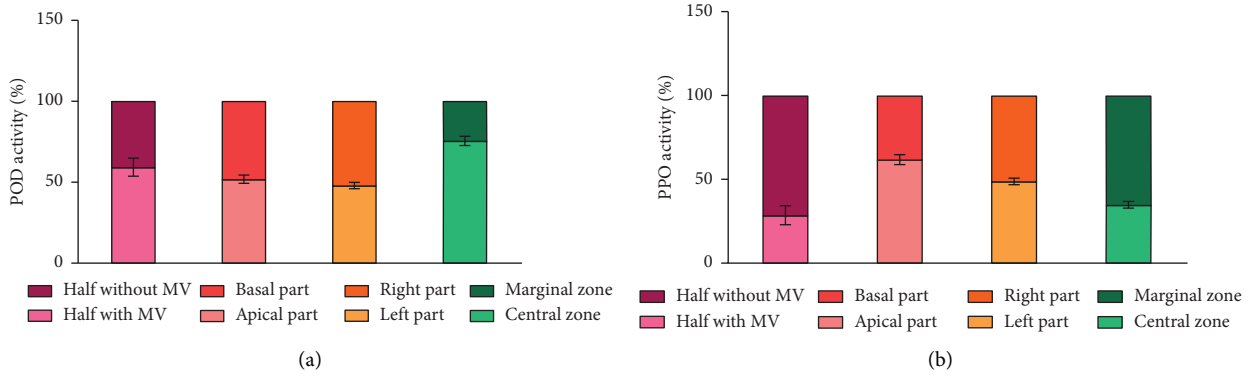

FIGURE 1: Spatial distribution analyses of POD (a) and PPO (b) enzymatic activities expressed as the percentage of values calculated on a fresh weight (FW) basis ($U{\cdot}[gFW]^{-1}$) in different areas of the leaf. Leaves were cut to obtain two halves, with and without midvein (MV), basal and apical part, right and left part, marginal and central zone. Mean values ± SD ($n = 3$) are reported.

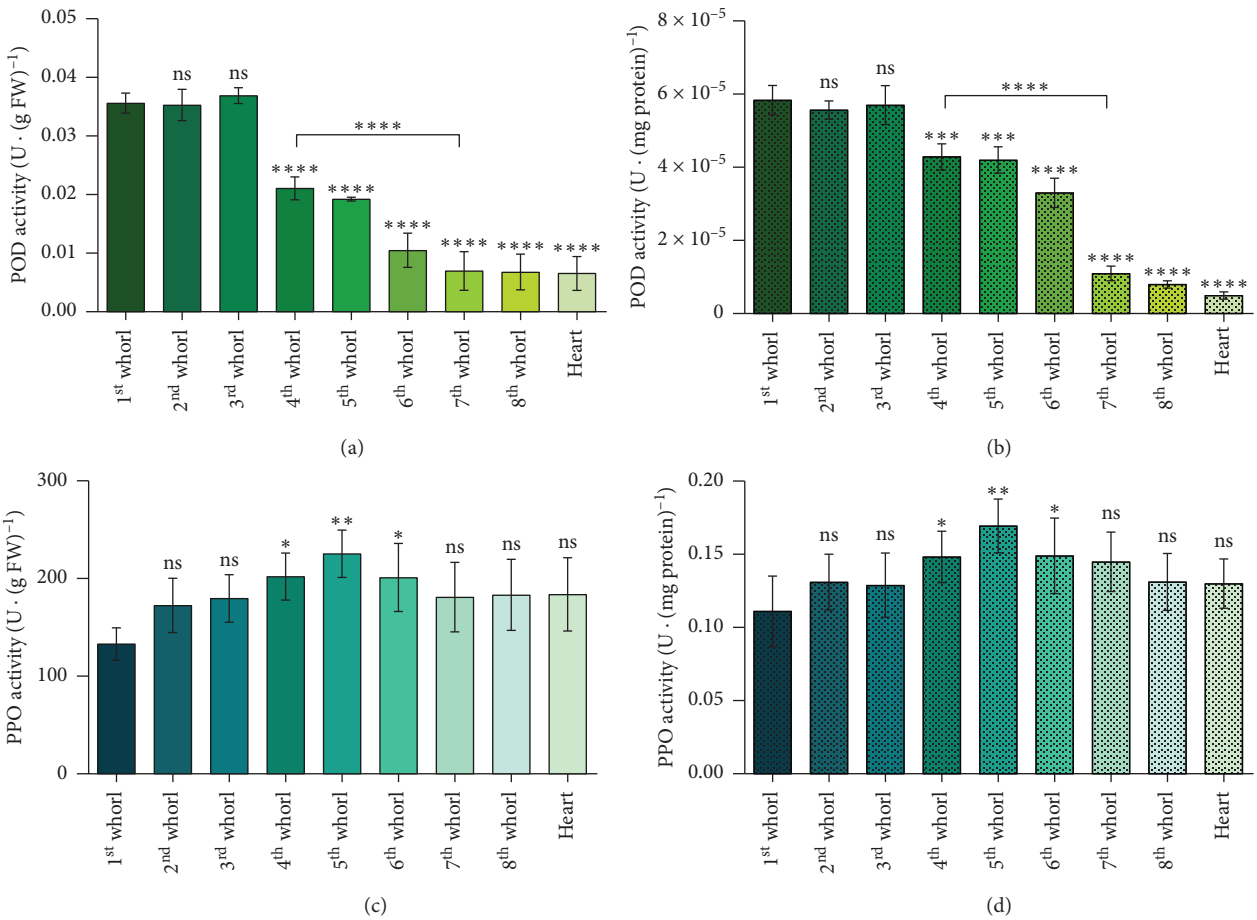

FIGURE 2: Spatial distribution analyses of POD (a, b) and PPO (c, d) enzymatic activities expressed on a fresh weight (FW) basis ($U{\cdot}[gFW^{-1}]$) (a and c) and on a total protein basis ($U{\cdot}[mg\ proteins]^{-1}$) (a and d) in all leaves of each whorl at T0. Significance levels are reported between the first whorl and each of the other whorls (a–d), and between the fourth and the seventh whorls (a, b). Mean values ± SD ($n = 3$) are reported. p levels have been calculated with one-way ANOVA; $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$; ns, not significant.

the browning phenomenon [50], the focus is placed on the contribution of POD activity to the development of lettuce browning in relation to postharvest processing involving cutting and cold storage.

3.3. POD Enzymatic Activity Increases During Storage at 4°C. To evaluate the role of POD in the browning phenomenon occurring in the postharvest phase of minimally processed lettuce, the temporal trend of POD enzymatic activity was analyzed after cutting lettuce leaves into pieces, which were subsequently stored at 4°C, for a time period of 20 days. Taking into consideration the results obtained from the analysis of POD spatial distribution in the whole lettuce head (Figure 2), the first, fourth, sixth, and seventh whorls were chosen for the subsequent analyses, as they were considered to be representative of the POD enzymatic activity's spatial trend. The temporal trend analysis highlighted a progressive increase in POD activity during storage, evident in all whorls analyzed between T0 and T20 (Figure 3). Interestingly, the ratio in POD activity levels between the outermost whorls and the innermost ones remained proportional during the time-dependent increment. Numerous studies have reported that high POD activity in plants mediates low-temperature stress tolerance through H_2O_2 elimination [51]. Indeed, environmental stresses, among which low temperature, generate excess amounts of reactive oxygen species (ROS), leading to oxidative stress [52], and antioxidant enzymes are considered the first line of defense to eliminate accumulated ROS. In this context, the increment of POD enzymatic activity after wounding and during storage at low temperature herein showed correlates with data previously reported by Cantos et al., in which a linear increment of POD activity upon storage after wounding was observed and has been correlated to a *de novo* POD synthesis [18]. These authors hypothesized that the induction of new POD could also be related to lignification processes to repair cell walls after tissue wounding [18].

3.4. Treatment With a Kiwi Peel Extract Hinders POD Activity Increase During Storage. The effect of the treatment with a crude kiwi peel extract on POD enzymatic activity was investigated during storage at 4°C over the 20-day period previously considered, in order to evaluate the possibility of using this approach at an industrial level to increase the shelf-life of RTE salads. Based on the results obtained from the temporal trend analysis of POD activity reported in Figure 3, and considering that the outermost whorl is generally discarded by the RTE industry, the fourth and the seventh whorls were examined as they were considered representative of higher and lower POD enzymatic activity. Data showed that the POD activity level, expressed both on fresh weight basis (Figures 4(a) and 4(c)) and on total protein content (Figures 4(b) and 4(d)), in treated leaves of both the fourth and seventh whorls is lower than the level observed in control leaves of the respective whorls. In T15 and T20, respectively, treated leaves compared to the respective control leaf showed a reduced increment in POD activity expressed in relation to total protein content, of about 32% and 33% in the fourth whorl, and of about 28% and 34% in the seventh whorl.

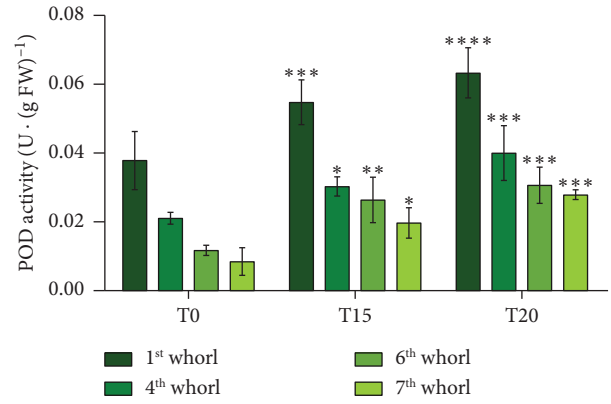

Figure 3: Time course analysis of POD enzymatic activity expressed on a fresh weight (FW) basis (U·(gFW^{-1})) in leaves from the first, fourth, sixth, and seventh whorls at T0, T15, and T20. Significance levels are reported between T0 and T15 or T20 for each whorl analyzed. Mean values ± SD ($n = 3$) are reported. p levels have been calculated with one-way ANOVA; $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

Since fruit peels, including kiwi peels, are rich in antioxidants [34, 39], it is possible to hypothesize that the effect of the treatment observed here in reducing the POD enzymatic activity is due to the antioxidant action of active compounds present in the crude extract, including ascorbic acid. In fact, having the potential to donate electrons as a coenzyme, ascorbic acid participates significantly in the removal of ROS under stress conditions. It is known that ROS production mediates POD enzyme induction [52], and since antioxidants such as ascorbic acid reduce ROS, it is possible to hypothesize that the antioxidant effect of the extract reduces the oxidative stress due to postharvest processing of lettuce, including wounding, and that this consequently causes a reduction in the induction of antioxidant enzyme levels, such as POD, here reported.

3.5. Treatment With a Kiwi Peel Extract Hinders Browning Increase During Storage. In order to quantify the browning development in sliced leaves of the fourth and seventh whorls during storage at 4°C, images of salad slices untreated or treated with the kiwi peel extract were captured and analyzed after 20 days (T20). For each condition, three sliced leaves were photographed and analyzed using ImageJ 1.44 software. RGB value measurements were carried out by drawing a ROI that includes the MV area of the transversal sections of the sliced leaf (Supporting Figures S1b and S2). Data showed that the treated leaves of both the fourth and seventh whorls accumulate less dark pigment on the cut surface of the salad slices than the control leaves of the respective whorl, as shown by the higher RGB values of the treated samples compared to the lower RGB values of the control samples (Figure 5).

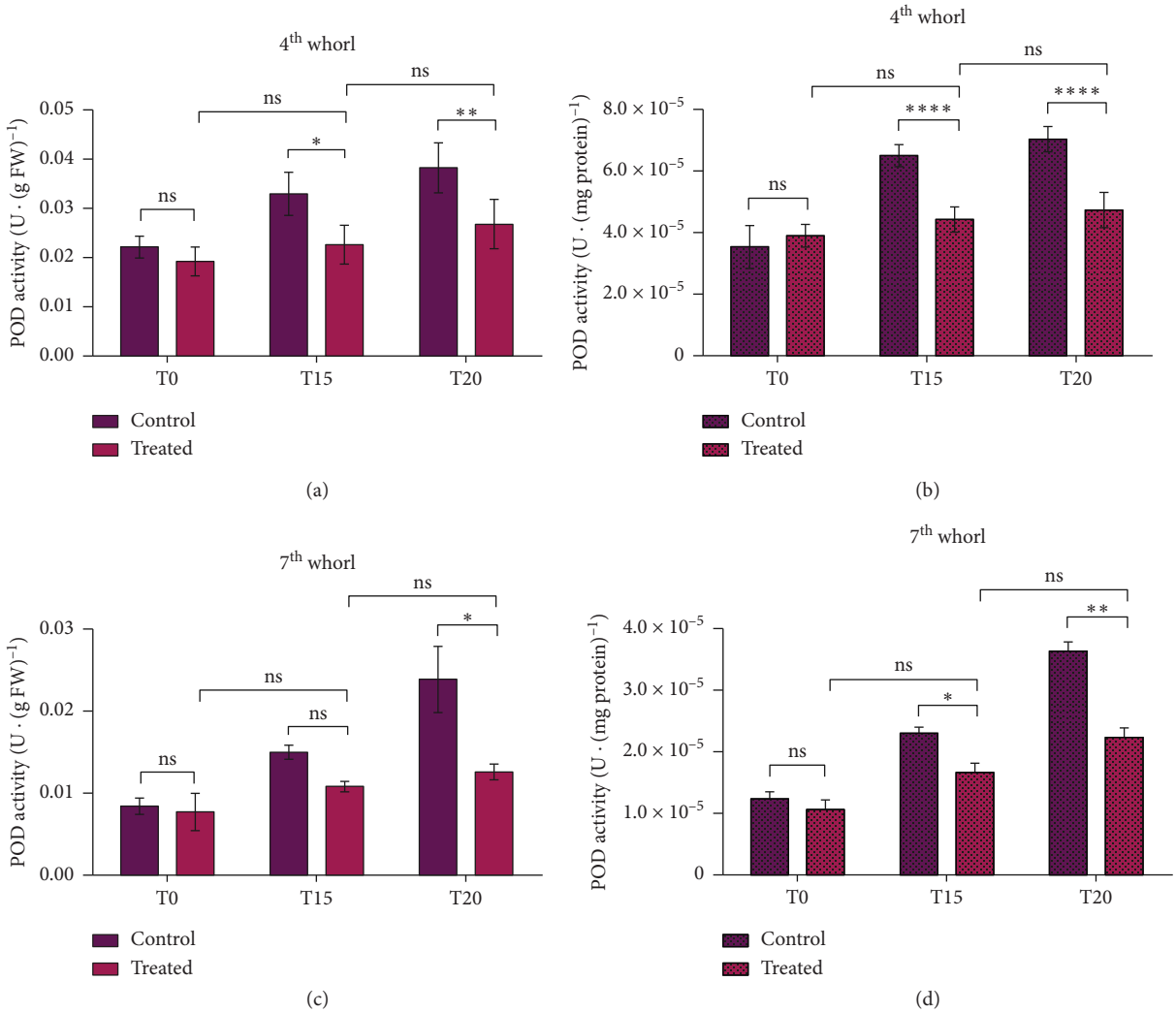

Figure 4: Time course analysis of POD enzymatic activity expressed on a fresh weight (FW) basis (U·[gFW^{-1}]) (a, c) and on total protein basis (U·[mg proteins]$^{-1}$) (b, d) in leaves from the fourth and seventh whorls treated or not treated (control) with the kiwi peel extract. Significance levels are reported between control and treated leaves for each time and whorl analyzed. Mean values ± SD ($n = 3$) are reported. p levels have been calculated with one-way ANOVA analysis; $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{****}p \leq 0.0001$; ns, not significant.

These data, together with the enzyme activity results shown in Figure 4, strengthen the hypothesis of a possible correlation between the increase in POD enzyme activity and the increase in browning predominantly in the cut area of the MV during storage in minimally processed lettuce leaves. The mechanism of action is yet to be elucidated, but it could be due at least in part to direct or indirect inhibition of POD enzymatic activity. This could be due to the high level of antioxidants in kiwi fruit peel [53] which alters the availability of H_2O_2, thus reducing the available substrate for POD activity. This hypothesis correlates with other antibrowning mechanisms reported, according to which antioxidant agents, including ascorbic acid, are able to prevent melanin formation by binding to intermediates of browning enzymatic reactions, thus detoxifying the oxidative stress generated by postharvest processes necessary to obtain RTE products [25]. Moreover, it is also possible that there could be an effect through a change in pH. In fact, it has been reported that the optimum pH of POD in Litchi pericarp was found to be around pH 6.5–7 and that POD activity is much reduced at lower pH levels [54]. Since fruit extracts are rich in organic acids [55], it is possible that treatment with kiwi fruit peel extract could lower the pH, thereby lowering the efficiency of POD enzymatic activity. Moreover, results confirm that treatment with natural extracts can be a valid alternative method to extend the shelf-life of RTE products [55]. This is crucial considering that, when seeking new strategies for developing antibrowning methods, it is necessary to take into account the needs of consumers in selecting safe and healthy products. The use of natural compounds, such as those derived from fruit and vegetable waste products as antibrowning agents would be of significant importance for a more widespread acceptance of these products by consumers. At the same time, the use of fruit and vegetable by-products as a source of antioxidant compounds represents a key solution in circular economy, thus contributing to the development of eco-sustainable strategies aimed at environment protection.

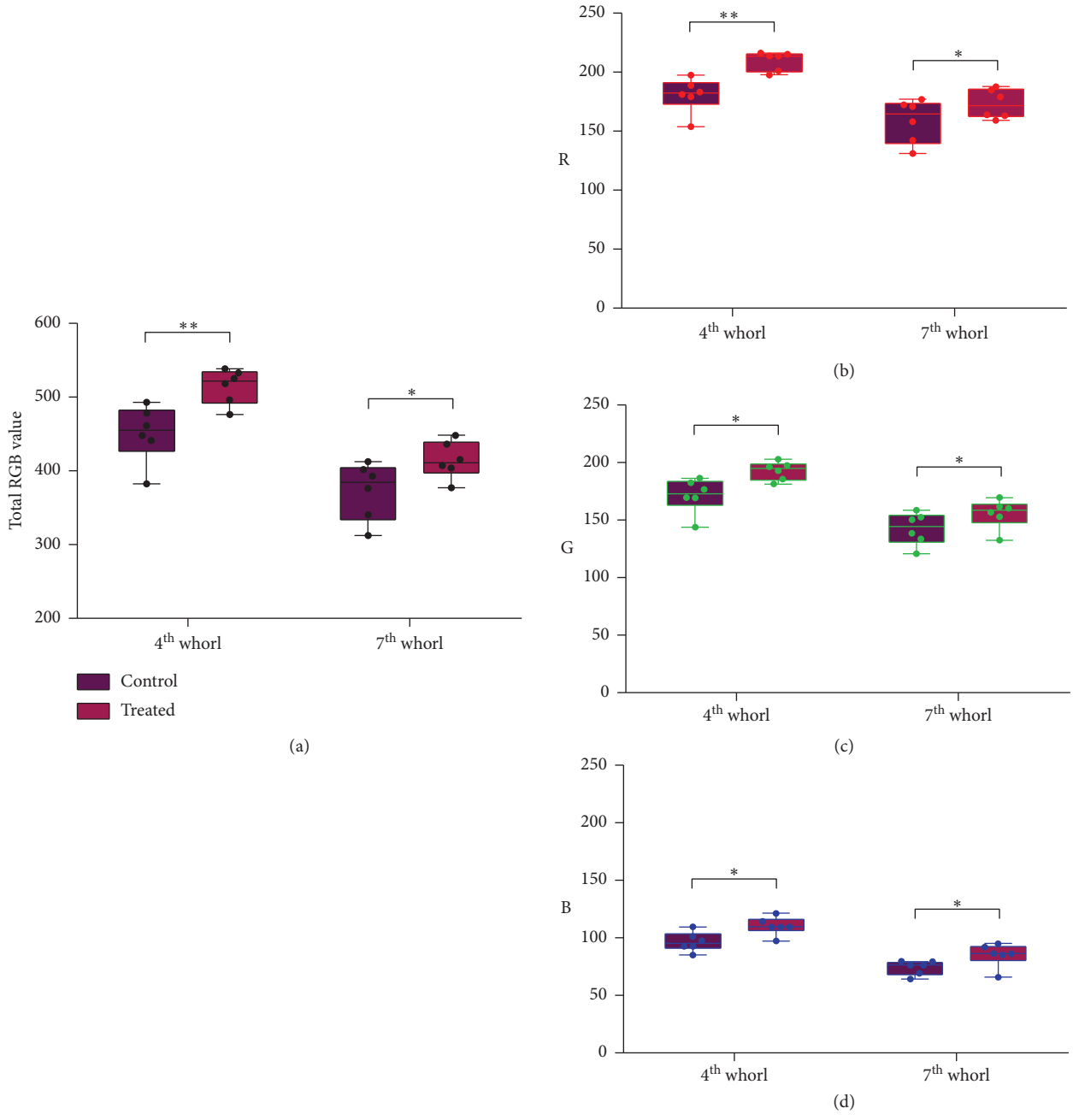

FIGURE 5: Quantitative analysis of the browning level in leaves of the fourth and seventh whorl untreated (control) or treated with kiwi peel extract during storage at T20. RGB value measurements were carried out by drawing a region of interest (ROI) that includes the MV area of the transversal sections of the sliced leaf and the total RGB values were used for determining the dark pigment formation on the cut surface of salad slices. Graphs show the total RGB value, considered as the sum of R, G, and B single parameters (a), and the R, G, and B values separately (b, c, d). Significance levels are reported between control and treated leaves of each whorl analyzed. Mean values ± SD ($n = 3$) are reported. p levels have been calculated with one-way ANOVA; $p > 0.05$; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

4. Conclusions

This work investigated PPO and POD enzymatic activities, which are mainly responsible for the browning phenomenon, in minimally processed lettuce, as well as the effect of a kiwi peel extract in reducing the enzymatic browning that occurs after processing RTE lettuce, in order to extend its shelf-life. Data herein reported demonstrated that the treatment of lettuce leaves with a kiwi peel extract significantly reduced the increase in POD activity over time, concomitantly inhibiting the development of browned areas. This evidence supports the hypothesis of a correlation between the increase in POD enzyme activity and the increase in browning predominantly in the cut area of the MV during storage in minimally processed lettuce leaves. Further work is required to develop this approach for industrial use,

assessing its use on greenhouse, field, and commercially grown material, and crucially ensuring the absence of enteric pathogens from the kiwi peel extract. Indeed, fruit peels are a known source of contamination [56], and specifically, pieces of evidence have been reported about contamination in kiwi fruit orchards and processing plants [57]. With this limitation in mind, however, the use of kiwi fruit peel has the potential to valorize plant waste and improve lettuce shelf-life, and it may also be applicable to other RTE salad species.

Data Availability Statement

The original contributions presented in the study are included within the article, and further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Supporting Figure 1: Representative images showing, respectively, how the leaves of both the fourth and seventh whorl were cut (a) as well as the transversal area of the MV section considered to carry out the analysis of the brown pigmentation for both control leaves and leaves treated with kiwi peel extract at Day 20 (T20; b). Supporting Figure 2: Representative images showing how the ROIs were drawn and analyzed using the ImageJ 1.44 software in order to quantify the browning pigmentation in both untreated leaves and leaves treated with kiwi peel extract during storage at 4°C.

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