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Article

Can Chitosan Applications in Pre- and Post-Harvest affect the Quality and Antioxidant Contents of Red Raspberries?

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Abstract: Red raspberry fruit production is increasing worldwide due to the growing consumer interest in foods with high antioxidant properties. However, raspberries are very perishable fruit with a short shelf life. Chitosan applications have shown promising results in promoting the storage of different berry fruit. This work aimed at analysing the effects of chitosan applied in pre- and/or post-harvest on the quality and antioxidant properties of raspberry fruit during the cold storage and room temperature conditions (i.e., 6 d at 4 °C and 3 d at 20 °C, respectively). Pre-harvest chitosan applications reduced fruit weight loss during cold storage and room temperature conditions but also reduced fruit decay at room temperature conditions. Furthermore, pre-harvest treatments with chitosan increased the total phenol and anthocyanin contents and promoted the highest total antioxidant activity with respect to other treatments. Of note, after the cold storage, post-harvest chitosan application drastically reduced the development of fungi that cause fruit decay, thus minimizing the potential risk of mycotoxin production. Overall, this study demonstrates that applications of chitosan in pre-harvest are sufficient to ensure the goal of maintaining and/or increasing the quality and antioxidant properties of fruit during cold storage and room temperature conditions.

Keywords: anthocyanins; antioxidants; ascorbic acid; fungal decay; pre-harvest factors and susceptibility; storage

1. Introduction

Red raspberry belongs to the *Rosaceae* family and is widely distributed in temperate regions of Europe, Asia, and North America. World raspberry production has grown about 80% over the last ten years. Indeed, from 2010 to 2019, the production has increased from 373 to 684 thousand tons [1]; the top 10 raspberry-producing countries are the Russian Federation, Poland, the United States of America, Serbia, Mexico, Ukraine, Spain, Chile, UK, and Bosnia Herzegovina. However, new producer countries such as Morocco or South Africa have strongly entered in the global raspberry market increasing competitiveness [2]. The increasing demand for these fruit is probably due to the growing consumer interest in foods with high antioxidant properties. Recent research supported the long-held beliefs that raspberries represent a particularly healthy food [3,4]. Indeed, fresh raspberry fruit constitutes a rich source of dietary fibre, vitamin C, and potassium. Moreover, raspberries are

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rich in phenolic compounds (e.g., anthocyanins, procyanidins, and ellagic acid), which are important for vascular health and cancer prevention [3,5–7].

Raspberries have a short post-harvest life (around 2–3 d from picking), mainly because of their loss of firmness and susceptibility to fruit rot, which limits their commercialization and consumption [8]. This implies that much of the harvested fruit is immediately distributed to the local markets to be sold fresh. Another part is frozen to prolong the post-harvest shelf-life, making them suitable for the worldwide market [9–11]. Furthermore, fresh fruit can be transformed into jams, syrups, juices, and distillates [12,13]. The different post-harvest storage conditions and the transformation processes working with other parameters (e.g., CO_2, O_2, temperatures, processing time) can strongly influence the antioxidant properties by altering the content in antioxidant molecules that possess health-promoting properties [3,14]. However, the shelf life of berries (whatever storage type is used) strictly depends on the mechanical injuries during the harvest or in the post-harvest processing, water loss, and fungal infections (e.g., *Botrytis cinerea*, *Alternaria* spp., *Mucor* spp., *Rhizopus* spp., *Penicillium* spp. and *Cladosporium* spp.) [15–21]. Over 20% of fruit and vegetables produced at the global scale are lost from post-harvest to the distribution [22], mainly due to microbiological spoilage. Therefore, it is necessary to develop decay-control measures that aim to maintain the quality of fruit and vegetables and protect against post-harvest diseases. Among the possible alternatives that can effectively reduce the post-harvest diseases in fresh fruit at both pre- and post-harvest stages, chitosan applications have shown promising results [23]. This natural polymer is a by-product of chitin, the second-largest renewable carbon source in the world after cellulose [24]. Chitosan is one of the most studied biomaterials thanks to its unconstrained biological properties like antimicrobial and plant growth regulatory activity, biodegradability, biocompatibility, and non-toxicity to humans [24–27]. It was the first substance approved by the European Union for plant protection (Reg. EU 2014/563) for organic farming and integrated management of plant diseases, thanks to its low toxicity. Chitosan has been widely used as a coating agent for various fruit, mainly for protection from post-harvest losses due to microbial infections [26–28]. Moreover, pre-harvest foliar spray applications of chitosan have been reported to increase the vegetative growth, yield, and secondary metabolites in plants [26]. Besides the numerous works conducted on different berry species (e.g., strawberry, blueberry), there are very few studies in the literature that have analysed the efficacy of chitosan in pre- and/or post-harvest on red raspberry fruits [17,23,29]. Therefore, this work aimed at increasing the knowledge of the potential positive influences of chitosan applied in pre- and post-harvest on the quality of raspberry fruit, by mimicking a realistic scenario of fresh raspberry fruit delivery (cold storage and room temperature conditions), focusing on its effects on fruit quality, fungal decay and fruit antioxidant properties.

2. Materials and Methods

2.1. Plant Material

The Raspberries of the primocane-fruit cultivar 'Glen Ample' were picked from a commercial orchard located in Abetone (PT), Tuscany, Italy (N 44 ° 08 ' 16 ", E 10°41'43", altitude 1349 m, annual average temperature: 1–19 °C). Three-year-old plants were cultivated on an espalier system with an anti-hail net and drip irrigation. The experiments were carried out in the summer growing season of 2020. Two rows were treated with chitosan, and two untreated rows represent the control.

The pre-harvest applications with chitosan (1%; v/v) were performed with a commercial formulation (Chitosan hydrochloride, Agrilaete, Italy), following the manufacturer's instructions. The chitosan solution was hand-sprayed over the plants until complete coverage. The treatments were applied at two fruit stages, when the raspberries were starting to turn pink and up to harvest (stages S2 and S4, respectively [30]; about 10 d passed between the two stages). Mature fruit were handpicked the day after the last application. The harvested raspberries were placed in plastic containers in an ice-cool box with ice sheets for transport to the laboratory.

At the laboratory, raspberry fruit from each treatment were selected for the absence of defects and uniformity of color and shape. Half of the total amount of chosen fruit treated with chitosan in

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pre-harvest (CH1) and untreated (C0) received a post-harvest application with chitosan (1% v/v; CH2 and C1, respectively) as follows (Table 1). Raspberries were dipped in the chitosan solution for 15 min and were allowed to dry on a thin plastic net placed on filter paper at room temperature (25 °C).

Table 1. Description of chitosan treatments carried out in pre- and post-harvest on red raspberries.

		Treatments
C0	At harvest	Untreated fruit (control)
CH1		Pre-harvest chitosan (1% v/v) treated fruit
C0	At the end of the experiment (6 d at 4°C + 3 d at 20°C)	Untreated fruit (control)
C1		Post-harvest chitosan (1% v/v) treated fruit
CH1		Pre-harvest chitosan (1% v/v) treated fruit
CH2		Pre- and post-harvest chitosan (1% v/v) treated fruit

Fruit of the four treatments (C0, C1, CH1, and CH2) were placed in macro-perforated PET (Polyethylene terephthalate) trays with a lid (9.5 × 14 × 4.5 cm). Fruit were stored for 6 d at 4 ± 1 °C and 70% relative humidity (cold storage), then exposed at 20 ± 1 °C and 70% relative humidity for 3 d (room temperature conditions). During the cold storage, fruit were weighed daily, whilst they were weighed at the end of room temperature conditions (day 9). The fruit decay index was performed after the cold storage (day 6) and at the end of the experiment (6 d at 4 °C + 3 d at 20 °C). Organoleptic characteristics (solid soluble content, titratable acidity, and color) and biochemical analyses (total phenol content, anthocyanin content, ascorbic acid content, and antioxidant capacity) were performed at harvest and at the end of the experiment, when the weight loss analysis showed the most significant differences among treatments (day 9). Results from biochemical analyses were expressed on a dry weight basis.

2.2. Fruit Quality Parameters

Weight loss was determined gravimetrically by weighing each plastic box at time zero and during the storage ($n = 3$) using a technical balance (Mod. PE 600, Mettler-Toledo S.p.A., Milan, Italy). Changes in fruit weight were expressed as a percentage of weight loss compared to the initial weight.

Five red raspberry fruit were squeezed by a press, and the juice was used to determine solid soluble content (SSC; $n = 3$) and titratable acidity (TA; $n = 3$). The SSC was measured by a refractometer (mod. with three scales ATC, Polsinelli S.r.l., San Giuliano Terme, Italy) and expressed as %. The titratable acidity was determined in 2 mL of juice sample diluted with 98 mL of distilled water, titrated with 0.1 M NaOH to pH 8.1 using a pH meter (XS Instruments, Modena, Italy). The results were expressed as g citric acid L^{-1}.

The surface color of each fruit was measured with a portable colorimeter (model CM-700d, Spectrophotometer, Konika-Minolta, Osaka, Japan) on the opposite sides of 15 fruit per treatment. Color was recorded using the CIE L^* a^* b^* uniform color space, where L^* indicates lightness, a^* indicates chromaticity from green to red, and b^* chromaticity from blue to yellow. Numerical values of L^*, a^*, and b^* were converted into hue angle (redness; Ho = arctan b^*/a^*), chroma [color saturation; Chroma = $(a^{*2} + b^{*2})1/2$], and XYZ coordinates [31].

2.3. Phenol Extraction and Analysis

About 0.1 g of fresh raspberry fruit powder, obtained by the milling of three fruit at low temperatures using a beater mill (SK 100 Cross Beater Mill, Retsch, Germany), from each treatment ($n = 3$) was homogenized with 1 mL of 80% (v/v) methanol solution by sonication for 30 min, keeping the temperature within the range 0 to 4 °C. After centrifugation (6,000 *g* for 10 min at 4 °C), supernatants were collected and passed through PTFE (Polytetrafluoroethylene; 0.20 µm pore size; Sarstedt, Verona, Italy). Extracts were stored at −80 °C before analysis.

Total phenol content (TPC) was evaluated according to the method reported by Dewanto [32] based on Folin–Ciocalteau reagent. Briefly, 5 µL of the phenolic extract was added to 120 µL of

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ultrapure H_2O and 125 µL of Folin–Ciocalteau reagent. The obtained solution was vigorously shaken and incubated for 6 min at room temperature. After the incubation, 1.25 mL of 7% $NaHCO_3$ was added, and then the solution was incubated for further 90 min at room temperature. The solution absorbance at 760 nm, using an Ultrospec 2100 Pro UV–VIS spectrophotometer (GE Healthcare Ltd., Chicago, IL), was recorded. Values were expressed as g gallic acid equivalent (GAE) kg^{-1}.

2.4. Total antioxidant activity analysis

Total antioxidant activity (TAA) was measured using the method reported by Brand-Williams et al. [33]. Briefly, 3 µL of the phenolic extract was added to 997 µL of a solution containing 3.12×10^{-5} M 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The decrease in absorbance at 515 nm was measured against a blank solution (without extract) after a reaction time of 30 min at room temperature using the Ultrospec spectrophotometer. Results were expressed as a percentage reduction of the initial DPPH absorption in the extracts and expressed as g Trolox equivalent (TE) kg^{-1}.

2.5. Total Anthocyanin Concentration

Total anthocyanin concentration (TAC) was determined using the pH differential method according to Giusti and Wrolstad, [34]. About 0.1 g of fresh raspberry fruit powder, obtained by the milling of three fruit at low temperatures using a beater mill, was extracted in acidified methanol (1% HCl, v/v) according to Siegelman and Hendricks [35], and kept overnight at room temperature (n = 3). The absorbance was recorded at 530 and 700 nm. The final absorbance (A_f) of diluted samples was calculated as follows:

$$A_f = (A_{530} - A_{700})_{pH\ 1.0} - (A_{530} - A_{700})_{pH\ 4.5}$$

Total anthocyanins were expressed as g of cyanidin-3-O-glucoside equivalents kg^{-1} (molar extinction coefficient of 34,300 M cm^{-1} [35], and molecular weight 484.8 g mol^{-1}).

2.6. Ascorbic Acid Concentration

The ascorbic acid concentration (AA) was measured according to Kampfenkel et al. [36] with some modifications. Extractions were carried out with the homogenization of about 0.1 g fruit powder, obtained by the milling of three fruit at low temperatures using a beater mill, with 1 mL 6% (v/v) trichloroacetic acid followed by centrifugation for 10 min at 10,000× g at 4 °C (n = 3). After the extraction, the assay was performed by adding 50 µL supernatant to 50 µL 10 mM dithiothreitol and to 100 µL 0.2 M Na-P buffer (pH 7.4). Samples were stirred and incubated for 15 min at 42 °C. Then, 50 µL 0.5% (w/v) N-ethylmaleimide was added, and samples were stirred again. After 1 min of stirring, 250 µL 10% (v/v) trichloroacetic, 200 µL 42 % (w/v) orthophosphoric acid, 200 µL 4 % (w/v) 2,2′-bipyridine (diluted in 70% (v/v) ethanol 70% (v/v) and 100 µL 3% (w/v) $FeCl_3$ were added to samples. After 40 min of incubation at 42 °C in a water bath, the increase in absorbance at 525 nm was measured against a blank [by using 50 µL of 6% (v/v) trichloroacetic acid instead of sample extract], a second reagent blank [by using 50 µL of 6% (v/v) trichloroacetic acid and 100 µL of H_2O instead of sample extract and 3% (w/v) $FeCl_3$, respectively] and a sample blank [by using 100 µL of H_2O instead of 3% (w/v) $FeCl_3$] to avoid possible anthocyanin interferences. All results were expressed as g ascorbic acid kg^{-1}.

2.7. Fruit Decay Evaluation

Fungal decay was visually inspected in raspberries for each treatment (20 fruit per replicate; n = 3). A fruit was considered infected when visible contamination was observed (development of mycelium on the fruit surface, brown spots, and softening of the injured zone). Decay severity was recorded according to an empirical scale with five degrees of fruit surface infection: 0 (healthy fruit), 1 (1 to 10%), 2 (11 to 25%), 3 (26 to 50%), and 4 (≥50%).

The McKinney index (MKI; [37], which considers both the incidence and severity of the decay, was calculated according to the following formula:

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$$MKI = [(\Sigma (d \times f) / (N \times D)] \times 100$$

where d represents the category of rot intensity scored on the fruit, f is its frequency, N is the total number of examined fruit (healthy and infected), and D is the highest decay intensity of the empirical scale. The MKI expresses the weighted means of the disease as a percentage of the maximum possible level. Microscopic examinations of fungi developed of the surface of raspberries were performed in order to identify them at the genus level according to Samson et al. [38].

2.8. Statistical Analysis

Data obtained from weight loss analysis were subjected to two-way ANOVA with treatment and time as sources of variation. Data obtained from SSC, TA, color, decay index, TPC, TAC, AA, and TAA analyses were subjected to a one-way ANOVA with treatment as the source of variation. All the means were separated by Fisher's least significant difference (LSD) post-hoc test ($P \leq 0.05$). The normality of data was tested using the Shapiro-Wilk test, while the homoscedasticity was tested using the Brown-Forsythe test. All statistical analyses were performed using GraphPad (GraphPad, La Jolla, CA, USA).

3. Results

3.1. Fruit Qualitative Parameters

The fruit weight loss analysis showed the first significative differences among treatments from day 5 (Figure 1), during the cold storage at 4 °C, in which CH1 treatments presented a lesser fruit weight loss than C0 fruit (-17%). This condition was maintained up to day 9, during room temperature conditions at 20 °C, when the most significative differences were detected (-22% for CH1 fruit with respect to C0 fruit; Figure 1).

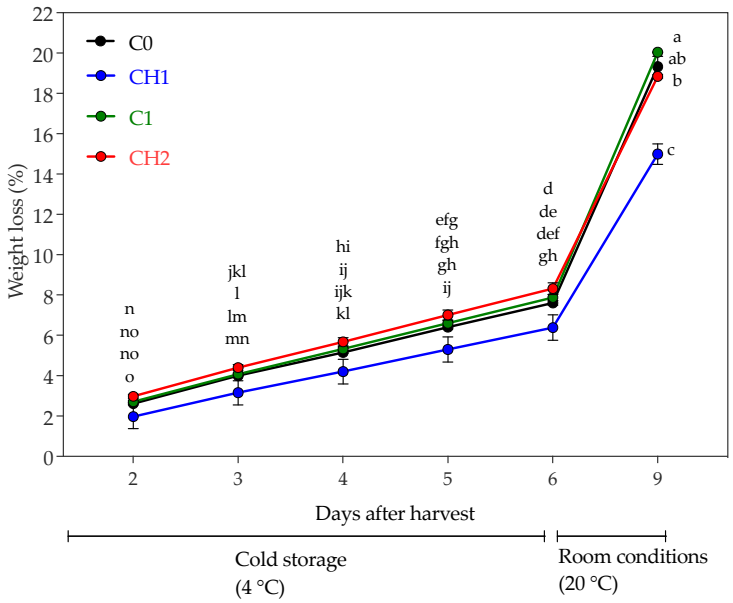

Figure 1. Red raspberries cv. Glen Ample weight loss (%), during 6 d of cold storage at 4 °C and after 3 d of room temperature conditions at 20 °C, analysed in control fruit (black line; C0), fruit with pre-harvest chitosan applications (blue line; CH1) control fruit with post-harvest chitosan application (green line; C1), and fruit with pre- and post-harvest chitosan applications (red line; CH2). Means ±SD (n=3), were subjected to two-way ANOVA with treatment and time as source of variation. Means flanked by the same letter are not statistically different for $P \leq 0.05$ after Fisher's least significant difference post hoc test.

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At harvest, no differences in SSC were observed between C0 and CH1 treatments, showing averaged values of 11.9% (Figure 2a). At the end of the experiment, only the post-harvest applications, C1 and CH2, showed no statistical differences from fruit analyzed at harvest (Figure 2a), while C0 and CH1 showed a reduction of 13 and 12%, respectively. No differences in TA values were observed either at harvest or at the end of the experiment among treatments (Figure 2b).

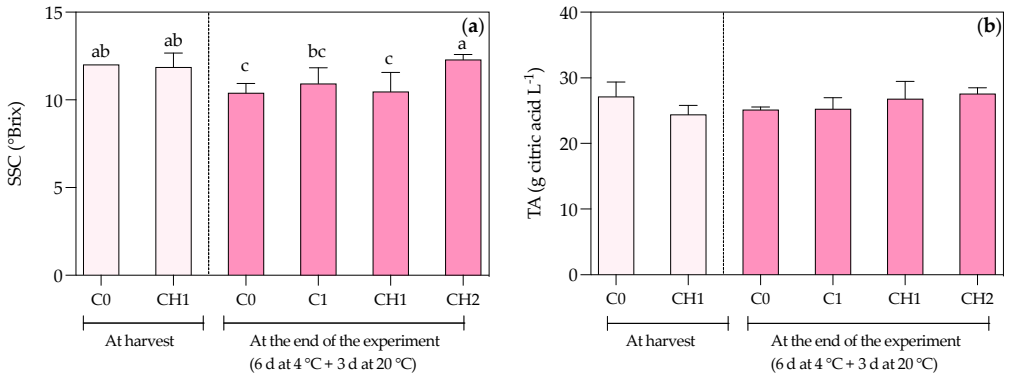

Figure 2. Soluble solid content (SSC; **a**), and titratable acidity (TA; **b**) in red raspberry fruit, analysed in control fruit (C0), fruit with pre-harvest chitosan applications (CH1) control fruit with post-harvest chitosan application (C1), and fruit with pre- and post-harvest chitosan application (CH2). Light-pink bars represent fruit analysed at harvest, while dark-pink bars represent those analysed at the end of the experiment (after 6 d of cold storage at 4 °C and 3 d of room temperature conditions at 20 °C; day 9). Means ±SD (n=3), were subjected to one-way ANOVA with treatment as source of variation. Means flanked by the same letter are not statistically different for $P \leq 0.05$ after Fisher's least significant difference post hoc test.

The CIE diagram showed that for fruit skin, x, and y coordinates fell in the pink region, independently of treatment and time (Figure 3). The colorimetric CIELab analysis performed on fruit skin highlighted that at the end of the experiment, values of L^* increased in CH1 and CH2 (15.5 and 11.1%, respectively) with respect to C0 (Table 2). At the end of the experiment, only fruit from the C0 treatment showed an increment in a^* (+42%) than C0 values detected at harvest (Table 2). No statistically significant differences in the b^* parameter among treatments were observed. In the fruit skin, at the end of the experiment, the Chroma parameter increased in C0 and CH1 (42 and 18.3%, respectively) with respect to C0 and CH1 values detected at harvest (Table 1). At the end of the experiment, a decrease in Hue values was observed only in CH1 and CH2 fruit (13.7 and 12.8%, respectively) compared to CH1 analysed at harvest (Table 2).

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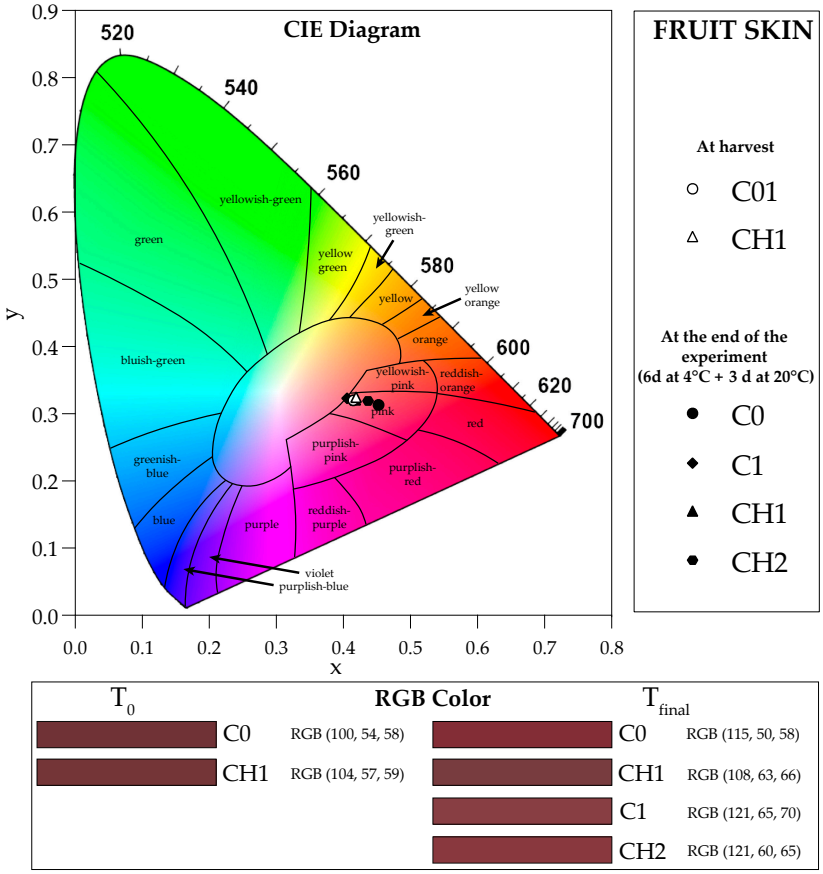

Figure 3. Chromaticity coordinates analysed in control fruit (open circle; C0), fruit with pre-harvest chitosan applications (open triangle; CH1) measured at harvest; and chromaticity coordinates of control fruit (closed circle; C0), control fruit with post-harvest chitosan application (closed rhombus; C1), fruit with pre-harvest chitosan applications (closed triangle; CH1) and fruit with pre- and post-harvest chitosan applications (closed hexagon; CH2) measured at the end of the experiment (after 6 days of cold storage at 4°C and 3 days of room temperature conditions at 20°C; day 9).

Table 2. CIELAB parameters of red raspberry (*Rubus idaeus* cv. Glen Ample) skin. Lightness (L*), redness (a*), yellowness (b*), chroma, and hue angle were measured at harvest in control and chitosan-treated in pre-harvest fruit (C0 and CH1, respectively), and at the end of the experiment (after 6 days of cold storage at 4°C and 3 days of room temperature conditions at 20°C; day 9), in control (C0), control treated with chitosan in post-harvest (C1), chitosan-treated in pre-harvest (CH1) and chitosan-treated both in pre-harvest and post-harvest (CH2).

		CIELAB parameters				
	Time	**L***	**a***	**b***	**Chroma**	**Hue**
C0	At harvest	28.45 ± 2.59^{b}	21.09 ± 3.74^{b}	6.32 ± 1.64	22.03 ± 4.04^{c}	17.73 ± 1.57^{b}
CH1		29.74 ± 1.57^{b}	21.33 ± 0.84^{b}	7.65 ± 0.33	22.67 ± 0.90^{c}	21.66 ± 0.25^{a}
C0	At the end of the experiment (6 d at 4°C + 3 d at 20°C)	29.95 ± 1.21^{b}	29.94 ± 2.63^{a}	8.97 ± 0.98	31.29 ± 2.78^{a}	17.96 ± 1.21^{b}
C1		32.14 ± 1.36^{ab}	20.35 ± 0.55^{b}	6.46 ± 0.44	21.37 ± 0.60^{c}	18.92 ± 1.40^{b}
CH1		34.60 ± 1.28^{a}	24.65 ± 2.51^{ab}	7.64 ± 1.96	26.81 ± 1.02^{b}	18.69 ± 0.64^{b}
CH2		33.28 ± 0.92^{a}	27.64 ± 7.05^{ab}	9.07 ± 3.46	18.51 ± 1.39^{bc}	18.89 ± 1.15^{b}

* Means ±SD (n = 3), were subjected to one-way ANOVA with cultivar as source of variation. Means flanked by the same letter are not statistically different for $P \leq 0.05$ after Fisher's least significant difference post-hoc test.

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3.2. Fungal Decay Evaluation

The evaluation of the fungal decay of raspberry fruit treated in pre- and post-harvest with chitosan and then stored at 4 °C for 6 d and 20 °C for 3 d was carried out. In general, the losses caused by fungal decay were not very high in the present study.

After 6 d of cold storage at 4 °C (Figure 4a), treatments with chitosan (C1, CH1, and CH2) significantly reduced the fungal damage with respect to C0 and, this reduction was very relevant when the fruit were treated in both pre- and post-harvest (CH2; Figure 4c). Moreover, the McKinney index values of postharvest treatments (C1 and CH2) were nearly half of C0 and CH1, respectively (Figure 4a). At the end of the experiment, only CH1 and CH2 showed lower McKinney index values than C0 (-59 and -73% for CH1 and CH2, respectively; Figure 4b).

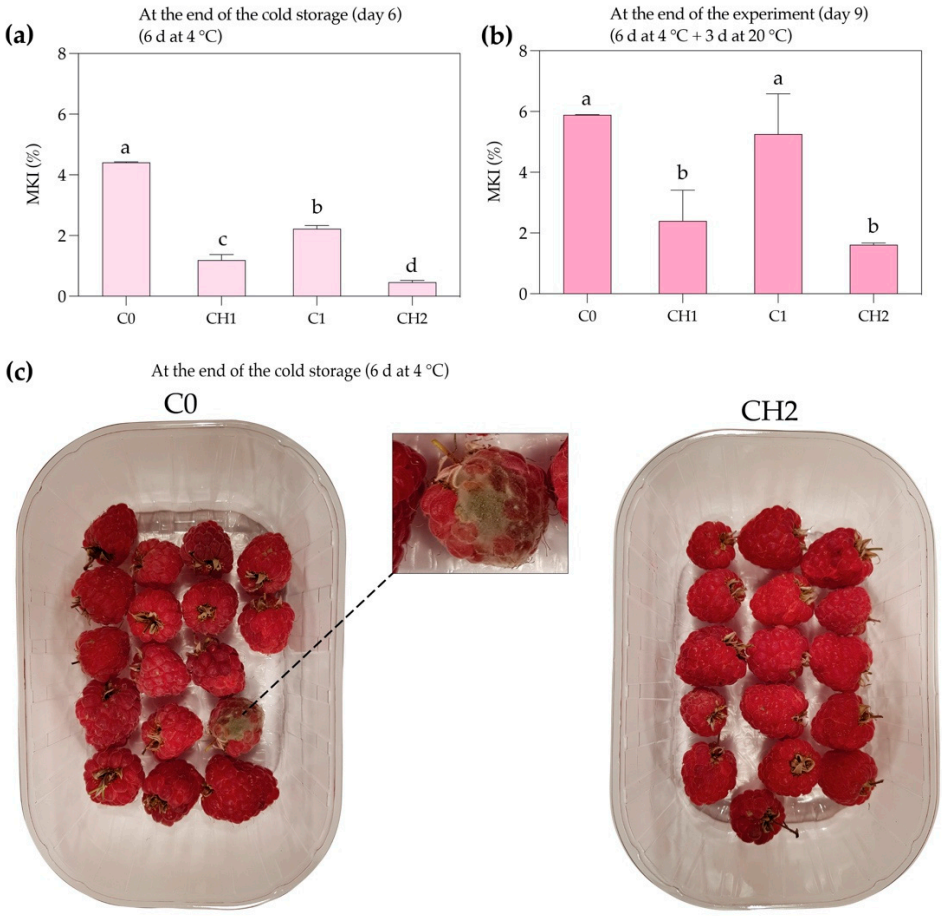

Figure 4. Fruit decay index (MKI) analysed in control fruit (C0), fruit with pre-harvest chitosan applications (CH1) control fruit with post-harvest chitosan applications (C1), and fruit with pre- and post-harvest chitosan application (CH2); measured after a cold storage of 6 days (**a**) and at the end of the experiment (after 6 days of cold storage at 4°C and 3 days of room temperature conditions at 20°C; day 9) (**b**). Light-pink bars represent fruit analysed after the cold storage, while dark-pink bars represent those analysed at the end of the experiment. Means ±SD (n=3), were subjected to one-way ANOVA with treatment as source of variation. Means flanked by the same letter are not statistically different for $P \leq 0.05$ after Fisher's least significant difference post hoc test. Fruit appearance of control (C0), and chitosan-treated fruit in pre- and post-harvest (CH2) after 6 days of cold storage at 4°C (**c**). In the highlighted box: red raspberry fruit decay caused by *Botrytis cinerea*.

3.3. Fruit Antioxidant Analyses

The total phenol content, assayed in fruit at harvest, is not different between C0 and CH1 fruit (9.87 ± 1.23 and 10.49 ± 1.39 g gallic acid eq. kg^{-1}, respectively; Figure 5a). At the end of the experiment

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(Figure 5a), TPC increased in C0 and CH1 fruit (40.8 and 67%, respectively as compared with C0 and CH1 analysed at harvest), reaching the highest values in CH1. On the other hand, no statistical differences with fruit at the harvest stage were observed for fruit treated with post-harvest applications (Figure 5a). At harvest, no significant differences were reported in fruit belonging to C0 and CH1 treatments (the concentration was about 2.24 g kg^{-1}), whereas at the end of the experiment, TAC increased only in fruit from CH1 and CH2 treatments (36.8 and 34.2%, respectively) with respect to fruit from C0 and CH1 treatments analysed at harvest. (Figure 5b). At harvest, AA content was unaffected by pre-harvest applications showing values of about 3.3 g kg^{-1} (Figure 5c). At the end of the experiment, AA content decreased in C0, C1, and CH1 by about 15% with respect to values obtained at harvest (Figure 5c). The capability of antioxidants assayed by DPPH method was not different among treatments measured at harvest (about 26.30 g Trolox eq. kg^{-1}; Figure 5d), while at the end of the experiment, fruit from CH1 and CH2 treatments showed higher values of antioxidant capacity than C0 (32.3 and 17.2%, respectively).

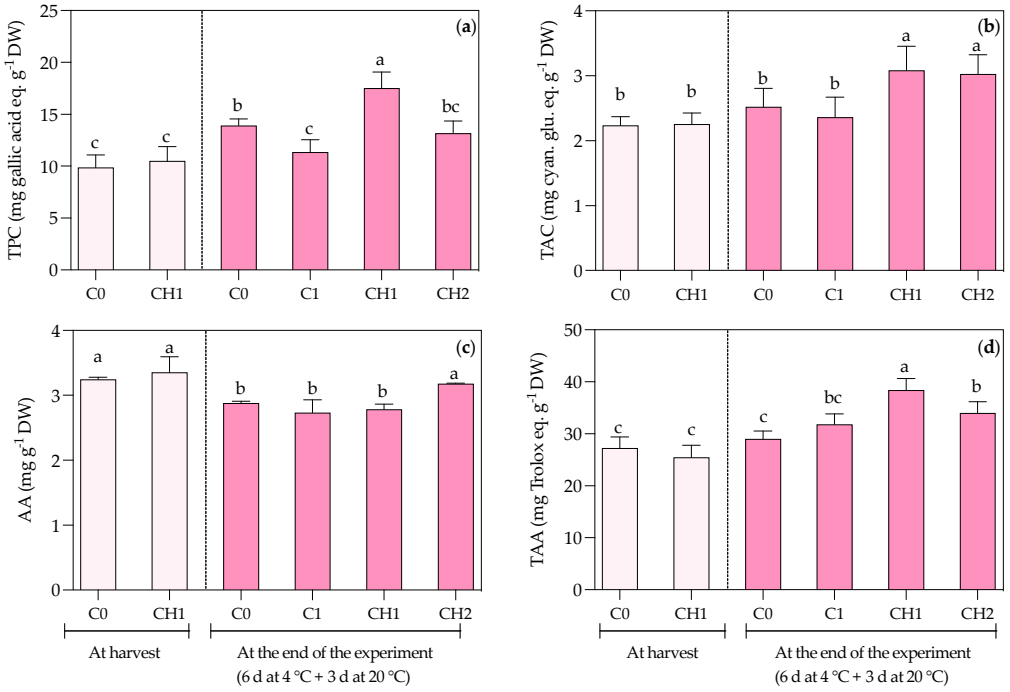

Figure 5. Total phenol content (TPC; **a**), total anthocyanin content (TAC; **b**), ascorbic acid content (AA; **c**) and total antioxidant activity (TAA; **d**) detected in control fruit (C0), fruit with pre-harvest chitosan applications (CH1) control fruit with post-harvest chitosan application (C1), and fruit with pre- and post-harvest chitosan application (CH2). Light-pink bars represent fruit analysed at harvest, while dark-pink bars represent those analysed at the end of the experiment (after 6 d of cold storage at 4 °C and 3 d of room temperature conditions at 20 °C; day 9). Means ±SD (n=3) were subjected to one-way ANOVA with treatment as source of variation. Means flanked by the same letter are not statistically different for $P \leq 0.05$ after Fisher's least significant difference post hoc test.

4. Discussion

4.1. Fruit Qualitative Analyses

Red raspberry fruit are a valuable and economically profitable nutritional crop due to the growing consumer interest in fruit with high antioxidant properties. However, raspberries have a short post-harvest life mainly due to a high fruit respiration rate and consequent loss of weight and

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firmness [39]. Indeed, these morphological and biochemical modifications can irremediably affect the fruit appearance and the antioxidant content, causing the depreciation up to the loss of product [14].

Only a few studies were conducted on the influence of chitosan sprays on raspberry fruits, and furthermore, as far as we know, only one study analysed the effects of treatments performed during the pre-harvest stage of fruits [17,23,29]. In our experiments, only chitosan applied in pre-harvest (CH1) positively influenced the fruit weight loss during the storage compared to other treatments. In Tezotto-Uliana et al. [23] chitosan application (1-2% v/v) performed at the pre- or post-harvest stage effectively delayed fruit respiration during the cold storage at 0 °C, retaining more key raspberry quality attributes than controls. However, the authors did not find changes in the weight loss parameter for all chitosan-treated fruit compared to controls. On the other hand, Han et al. [29] found that chitosan post-harvest application (2%; v/v) contributed to reducing fruit weight loss during the cold storage at 2 °C. Similar results observed in CH1 samples were also reported in the grape [40], muskmelons [41], and peaches [42,43]. This phenomenon can be explained by the fact that pre-harvest applications of chitosan may have partially inhibited the polygalacturonase activity in cells extending the cell wall integrity [44,45].

The lack of a positive effect of both chitosan post-harvest treatments (C1 and CH2) on fruit weight loss can probably be explained by two reasons: i) in C1 treatments, the lower chitosan concentration (1%; v/v) and the higher storage temperature (4 °C) utilized in our experiments with respect to Han et al. [29], affected the post-harvest treatment efficacy by reducing the moisture barrier property of chitosan; ii) in CH2, the double application of chitosan (in pre- and post-harvest), limiting the fruit respiratory gas exchange (O_2 and CO_2), could have favoured and accelerated fruit senescence, generating anaerobic fermentation products, which probably affected the fruit cell to cell adhesion and fruit cell wall stability [46–49].

SSC and TA are important parameters to assay fruit quality [9]. In our experiments, no differences were found in TA among treatments. From the literature, the fruit coating with chitosan preserved SSC and TA attributes by reducing fruit metabolic activities due to reduced ethylene production during the storage [23,50,51]. The preservation of SSC observed in our experiments during the storage only in fruit treated with chitosan at post-harvest (C1 and CH2), suggests that respiratory processes were slowed with post-harvest chitosan applications [51].

The skin color can influence the consumers' perceptions about the ripeness and freshness of red raspberry fruit. According to the CIE XYZ model, neither the different applications (pre- and/or post-harvest) nor storage had significantly influenced the chromaticity coordinates (x and y). Palonen and Weber [52] found a decrease in the L^* a^* b^* parameters during storage at both 1 and 7 °C, indicating darkening of berries in accordance with previous findings [9,53]. This was not the case in our study, in which L^* a^* b^* values slightly increased or were not statistically different from values detected at harvest. The pre-harvest coating treatment led to an increase in the brightness of samples and, at the end of the experiment, the fruits treated in the pre-harvest stage showed significantly higher brightness values than the others. This was likely due to changes in the surface reflection properties during storage, which resulted in this increase in brightness. However, overall, the small variations in CIE L^* a^* b^* color space parameters suggested that fruit skin color changed only slightly during storage.

4.2. Fungal Decay on Treated and Untreated Raspberries

Cold conservation and the use of chitosan in pre- and post-harvest have been used alone or in combination to keep the quality of fresh fruit, reducing the development of microorganisms that cause rotting [17,23,29,54]. Other authors found that raspberries stored at0, 5 or 10 °C showed slight signs of decay that become more evident in fruit stored at higher temperatures (15 or 20 °C) [54]. The combination of lower temperatures and chitosan can be successfully used to delay the germination of spores of food spoilage fungi and, therefore, can be used to extend the shelf life of food. Indeed, in *Aspergillus niger*, the combination of low temperature and chitosan is synergistic in reducing spore germination, whereas no synergism was observed when chitosan was used at temperatures >18 °C [55]. In light of these considerations and in our experimental conditions, chitosan application to

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raspberry plants in the field (pre-harvest treatment) seems to be very important in the control of fruit decay at the end of the experiment (4 and 20 °C for 6 d and 3 d, respectively), since only pre-harvest applications were able to control the fungal decay in the days following cold storage. Similarly to what was observed in this research, [56] reported that chitosan pre-harvest sprays significantly reduced post-harvest fungal rot and maintained the quality of strawberry fruit compared with control.

In our experiments, considering the cold storage period alone, a synergistic effect has been observed between chitosan and low temperature and, in this case, not only the treatment with chitosan in pre-harvest but also that in post-harvest had a good effect on the reduction of fungal decay. Moreover, microscopic observations carried out on the fruit that showed decay revealed the presence of different fungal genera such as *Alternaria*, *Cladosporium*, *Botrytis*, *Epicoccum*, *Colletotrichum*, *Mucor* and *Penicillium* (data not shown). Some of these genera included many mycotoxigenic species as reported elsewhere, [57] and for this reason, post-harvest treatment with chitosan is important to reduce the fungal inoculum present on the raspberry fruit.

4.3. Fruit Antioxidant Properties

Red raspberry fruit are characterized by antioxidant properties thanks to their good content in polyphenols and ascorbic acid. In particular, raspberries are rich in flavonoids such as anthocyanins [cyanidin 3-O-glucoside and cyanidin 3-O-sophoroside; and ellagitannins, with lambertianin C and sanguiin H-6 as the most representative of this group [3,58]. All these antioxidant molecules make red raspberries a healthy addition to a well-balanced diet. In our experiments, the TPC values observed in fruit at harvest were lower (about 50%) than those found in the literature for fresh Glen Ample raspberries [59,60]. It is well known that polyphenol content in raspberry fruit is strongly influenced by environmental conditions (e.g., light, temperature, and soil conditions) [60,61], which may explain the high variations in TPC with other reports.

The different storage environments can strongly influence the content of antioxidant molecules in red raspberries [14]. In our experiments, the further chitosan application in the post-harvest stage did not alter the TPC at the end of the experimental conditions, remaining unchanged with respect to C0 and CH1 analysed at harvest. Similar results were reported for litchi fruit [62], in which the post-harvest treatment with chitosan delayed the changes in concentrations of total phenols during storage. Moreover, also in a work conducted on grape berries, Sabir et al. [63] found that chitosan treatments were effective in maintaining the initial phenol content and many other metabolites. Therefore, we suggest that the unchanged TPC variations with respect to fruit at harvest, in post-harvest chitosan-treated fruit, indicated that chitosan coatings reduced the external environmental influences on total phenol concentrations.

Anthocyanins are water-soluble phenolic compounds belonging to the flavonoid group. They are plant pigments responsible for the red, pink, purple, and blue colors in plant tissues [64]. In red raspberries, most anthocyanin molecules are derived from cyanidin (one of the six known anthocyanidins or deglycosylated anthocyanin structures; [58]. The increment of TAC observed at the end of the experiment only in pre-harvest treated fruit, suggests that pre-harvest chitosan applications induced late responses in the secondary fruit metabolism during the storage period upregulating the anthocyanin biosynthesis. Indeed, besides the effects of chitosan on fungal diseases [17], it was also reported that chitosan treatments elicit a series of plant defense mechanisms correlated with enzymatic activities [26,65]. In strawberries treated with chitosan, Landi et al. [66] reported that chitosan up-regulated genes involved in the phenylpropanoid pathway such as, chalcone isomerase (CHI), flavonol synthase (FLS) and anthocyanidin synthase (ANS). Moreover, after chitosan treatments, similar results were also observed in grape berry skins at ripening, with the upregulation of chalcone synthase (CHS), and UDP-glucose-flavonol 3-O-glucosyl transferase (UFGT) [67]. CHS, CHI, ANS, and UFGT are key downstream enzymes for the synthesis of anthocyanins [68,69]. The fact that the increased TAC did not result in a higher TPC content in CH2 fruit may be due to a different re-arrangement of the concentrations of other phenols.

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The retention of AA reported only for the fruit treated both in pre- and post-harvest, could suggest that during the storage, the double chitosan treatment reduced the O_2 available for oxidative reactions by coating the fruit, resulting in better AA retention. However, if this hypothesis could explain better AA retention in stored fruit such as strawberries [51,70] or other fruit [71–73], previous research on chitosan coating in raspberries showed contrasting results. Indeed, Tezotto-Uliana et al. [23] reported adverse effects of chitosan applications in AA retention during storage, while Zhang et al. [17] found a beneficial effect of chitosan coating on AA content. Therefore, future experiments focusing on the molecular relations between AA and chitosan application on red raspberry fruit should be conducted to fully elucidate the apparently conflicting results.

It was reported by Beekwilder et al. [6,74] that anthocyanin and ascorbic acid contents together can contribute more than 40% to the total antioxidant activity in red raspberries. The antioxidant activity of anthocyanins is linked to their molecular structure: the kind, number, and position of substituents and the sugar moiety bonded on the flavylium ion [75,76]. On the other hand, AA is a water-soluble ketolactone with two hydroxyl groups. When oxidized, it converts to the ascorbate radical (Asc•-) and then spontaneously to the dehydroascorbic acid; however, the radical form is stable enough and quickly turns to dehydroascorbic acid (the stable compound), and this makes ascorbic acid an excellent radical scavenger [77]. Therefore, it is reasonably conceivable that at the end of the experiment, the higher anthocyanin recorded in CH1 and CH2 fruit, and the better retention of AA observed in CH2 fruit promoted the TAA with respect to controls. The pre-harvest chitosan applications have proven to be the most effective in promoting the TAA in red raspberries. From the obtained results, field applications of chitosan are preferable in pre-harvest to achieve the goal of maintaining and/or increasing the fruit antioxidant properties.

5. Conclusions

In summary, chitosan applications during pre- or post-harvest in red raspberry differently affected both fruit qualities and antioxidant properties. Chitosan treatments only in the pre-harvest stage reduced fruit weight loss during the whole cold storage/room temperature conditions, reduced the fungal decay, increased the total phenol and anthocyanin content, and increased the antioxidant activity with respect to other applications. Moreover, the post-harvest application of chitosan drastically reduced the development of fungi that cause fruit decay after cold storage, thus minimizing the potential risk of mycotoxin production. The data obtained in our study, for red raspberry fruit, suggest that the use of chitosan applications in pre-harvest can effectively preserve the fruit quality and antioxidant properties during storage.

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