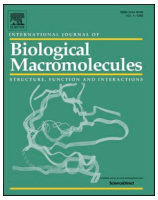
International Journal of Biological Macromolecules 275 (2024) 133149

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



Insect-derived chitosan, a biopolymer for the increased shelf life of white and red grapes

Anna Guarnieri [a], Micaela Triunfo [a], Dolores Ianniciello [a], Francesco Tedesco [c], Rosanna Salvia [a,b,*], Carmen Scieuzo [a,b], Eric Schmitt [d], Angela Capece [c], Patrizia Falabella [a,b,*]

[a] Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
[b] Spinoff XFlies s.r.l, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
[c] School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy
[d] Protix B.V., Industriestaat 3, NC 5107 Dongen, the Netherlands

ARTICLE INFO

Keywords:
Hermetia illucens
Chitosan coating
Postharvest fruit protection

ABSTRACT

Post-harvest water loss and microbial infections are the root cause of the rapid deterioration of fresh fruit after the picking process, with both environmental and economic implications. Therefore, it is crucial to find solutions that can increase the shelf life of fresh fruits. For this purpose, edible coatings, naturally derived and non-synthetic, are acknowledged as a safe strategy. Among polymeric coatings, chitosan is one of the most effective. In this work, this biopolymer, produced from chitin extracted from *Hermetia illucens*, an alternative and more sustainable source than crustaceans (the commercial one), was exploited to extend the shelf life of white and red grapes. Chitosan from *H. illucens* pupal exuviae, at 0.5 % and 1 % concentrations, was applied on both grapes, which were then stored at room temperature or 4 °C. The study of chemical-physical parameters such as weight loss, Total Soluble Solids and pH, demonstrated the effectiveness of the biopolymer, even better than crustacean chitosan. Moreover, the analysis of nutraceutical properties has demonstrated that this natural edible coating improves the quality of grapes, with beneficial effects for human health. The obtained results, therefore, confirmed the viability of using insect-chitosan as an alternative to crustaceans for the preservation of fresh food.

1. Introduction

In recent decades, the increased awareness of the harmful effects of chemical abuse, along with the improvement in the living standards of humans, has led to a growing demand for fresh biological fruits. Among them, grapes are one of the most popular and widely consumed fruits worldwide [1,2], arousing great commercial interest due to their excellent flavor and taste properties, as well as their high content of metabolites that ensure their optimal nutritional value [3].

The high concentration of phenols grants grapes excellent antioxidant properties. Grapes are also rich in anthocyanins, natural pigments, and resveratrol, having valuable anti-cancer, anti-inflammatory and antibiotic qualities [2,4,5]. This fruit belongs to the category of "non-climacteric" fruits, and it is sometimes affected by physiological changes that result in its spoilage [6].

During the storage period, indeed, the fruit can undergo softening, weight loss, or detachment of the berry [6,7], as well as browning, appearance of bacterial infections, wilting of the peduncular structures, and abundant water loss [1,8,9]. Indeed, it is particularly complicated to maintain the preservation of the fruit and, therefore, several solutions have been sought to prevent its spoilage and, consequently, its waste [8–10]. Among the measures to prevent grape spoilage and to also ensure quality during the winemaking process, low-temperature storage and sulfur dioxide usage are the most widespread [8,11]. Nevertheless, the latter can have some negative effects, as it compromises fruit original flavor and may result in damage from peel bleaching and rachis discoloration, as well as can lead to serious health risks [12,13]. One of the safest, most popular, and effective solutions is to preserve fresh food in dedicated packaging designed to maintain its freshness characteristics and organoleptic properties, thus increasing its shelf life and also customer satisfaction. However, a major limitation is that most of the packaging currently used is typically composed of plastic, resulting in significant negative impacts on the environment [14–16]. Therefore, research is increasingly focusing on the use of natural coatings to prevent the spoilage of fresh food [17–21]. Specifically, edible coatings based on biomaterials represent a highly effective and low-cost method

* Corresponding authors at: Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.
E-mail addresses: r.salvia@unibas.it (R. Salvia), patrizia.falabella@unibas.it (P. Falabella).

https://doi.org/10.1016/j.ijbiomac.2024.133149
Received 30 January 2024; Received in revised form 5 June 2024; Accepted 12 June 2024
Available online 30 June 2024

to prevent spoilage of fresh fruit [22–27]. Edible coatings can retard dehydration, arrest respiration, and improve the retention of volatile flavor compounds, thereby reducing pathogen attacks and microbial growth [28,29]. Among bio-based edible materials, natural polymers, due to their high selective-permeability coefficient (CO_2/O_2) and partial moisture barrier, can maintain stable transpiration and respiration rates [30]. They are also able to preserve structural and mechanical characteristics of the product, helping to retain its texture and its consistency [31], and they can be applied as coating on the surface, or turned into films [32]. One of the most interesting and effective natural polymers is chitosan [27], thanks to its remarkable properties [33], most notably antimicrobial [34–40] and antifungal ones [41–44]. Furthermore, besides the antifungal activity tested on various fruits [45–50], chitosan is also able to prevent the eventual depigmentation and, therefore, the browning of the fruit [27]. The limitation in the use of chitosan as a film is related to its poor stability at acid pH [51] and, for this reason, it can be functionalized with other materials to improve its properties [52].

The biopolymer derives from the alkaline deacetylation of chitin [53–56]. Using a chemical treatment method, chitin is extracted from *H. illucens* pupal exuviae via acid demineralization and sodium hydroxide deproteinization [57]. After these two steps, an optional decoloration phase could be performed, yielding both decolorized and not decolorized chitin, and the respective chitosan, having a different degree of purity and specific physico chemical features, that can be functional for the different applications of the biopolymer [55]. Chitin is mainly extracted, at industrial and commercial scale, from the exoskeleton of crustaceans [56].

The wide range of applications of chitosan has led to the exploration of alternative and more sustainable sources [58–63]. Among these, insects, and specifically the bioconverter *Hermetia illucens*, is arousing great resonance. *H. illucens* larvae feed on organic waste of different origin, converting it into larval biomass, rich in fats, proteins, chitin and antimicrobial compounds [58,64–67]. Pupal exuviae, a waste product deriving from insect-breeding, represent the biomass with the highest chitin content, that can be thus recovered and exploited to satisfy the market demand for chitin and chitosan [55,68,69].

The purpose of this work was to investigate, for the first time, the effect of chitosan-based coating produced from the pupal exuviae of *H. illucens* on the shelf life of white and red grapes. The novelty of the work is to valorize insects as a viable innovative and alternative source of chitosan, particularly starting from pupal exuviae, a waste product of *H. illucens* growth cycle.

2. Materials and methods

2.1. Materials

Pupal exuviae of *H. illucens,* provided by the manufacturer Protix (Dongen, The Netherlands), were the raw material used as starting substrate for obtaining chitosan. This biopolymer was supplied by Xflies s.r.l. (Potenza, Italy). All chemicals used in the experiments (sodium hydroxide, acetic acid, glycerol, tween-80, methanol, Folin–Ciocalteau, gallic acid, sodium nitrate, aluminum chloride, quercetin, potassium chloride, sodium acetate, ABTS, Trolox), including commercial chitosan (K), were purchased from Merck KgaA (Darmstadt, Hesse, Germany). White and red grapes were supplied from a local producer (APOFRUIT Italia soc. coop. agricola, Scanzano Jonico, Matera, Italy).

2.2. Fruit preparation

The berries were separated from the whole bunch, in order to be processed individually. They were selected based on their similar size, shape, color and degree of maturation, as well as the absence of fungal and visible mechanical damage.

2.3. Chitosan production and characterization

Pupal exuviae of *H. illucens* were exploited as substrate for chitin extraction. Decolorized (Dec) and not decolorized (No Dec) chitosan were produced by heterogeneous deacetylation, as reported in Triunfo et al. [55]: No Dec and Dec chitin were suspended in 12 M sodium hydroxide at 120 °C; the deacetylated chitin was neutralized, solubilized in 1 % acetic acid and then precipitated to obtain a high-purity chitosan. Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analyses were performed to verify the purity of polymer, as reported in Triunfo et al. [55,69]. The deacetylation degree (DD), and the viscosity-average molecular weight (M_v) of No Dec and Dec chitosan were determined, in accordance with Triunfo et al. [69,70].

2.4. Preparation and application of chitosan coating solutions

In order to obtain chitosan coating solutions, the polymer samples, including both commercial (K) and insect-derived (No Dec and Dec chitosan), were dissolved in a 1 % (*v*/v) acetic acid solution. Then, 2 % (v/v) glycerol and 0.2 % Tween-80 were added (hereafter defined as solvent). Two different concentrations of each chitosan solution were prepared: 0.5 % and 1 % (*w/v*). Grapes without treatment (Ctrl -) and grapes treated with the solvent alone were used as controls. The kinematic viscosity of each coating solution was assessed, and its pH was adjusted to prevent precipitation at alkaline pH levels [57]. Using the spraying method, all chitosan coating solutions were applied on white and red grapes using an aerograph (Martellato s.r.l., Rovigo, Italy). The spraying of the fruit was performed twice, at room temperature (RT), to ensure a uniform coverage of the fruit surface with the coating solution. Evaluation of the effectiveness of the chitosan coating on the grapes was carried out under two storage conditions: room temperature and 4 °C, storing the fruit until decay.

2.5. Assessment of physico-chemical properties of white and red grapes

2.5.1. Weight loss (WL)

Weight loss was assessed by measuring the grape weight, both at time zero (T0) and after storage. An electronic balance (Sartorius-BCE ENTRIS II, Göttingen, Germany) was used for weight evaluation. The quantified parameter was expressed as the percentage loss of the initial fresh weight.

2.5.2. Total Soluble Solids (TSS)

The standardized EN ISO 2173:2003 method enabled the evaluation of Total Soluble Solids (TSS) content using a digital refractometer. By analyzing the fruit pulp at T0, the variation of this parameter during the storage period was determined and expressed as Brix°.

2.5.3. pH

The pulp pH was measured, at RT, with a pH meter (Orion Research Inc., Boston, USA). The parameter was analyzed by evaluating its variation throughout the storage period.

2.6. Extraction and quantification of total phenolic and total flavonoid compounds

White and red grape extracts were prepared according to the method reported by Triunfo et al. [69]. Briefly, an 80 % (*v*/v) methanol solution was prepared and the pulp was then dissolved in the solution. The samples were sonicated and stirred for 1 h. Afterwards, the resulting mixture was separated on filter paper, and the supernatant was centrifuged for 10 min, at 4 °C and 5000 g. On each sample, the extraction procedure was performed twice. The total supernatants were employed for the determination of the Total Phenolic and Flavonoid Content and Total Antioxidant Activity.

2.6.1. Total Phenolic Content (TPC)

Total Phenolic Content (TPC) of both white and red grape fruits was determined using the Folin-Ciocalteu reagent, according to the method described by Triunfo et al. [69]. The measurement of absorbance at 723 nm after 1 h of incubation was used to quantify TPC, reported as mg gallic acid equivalent (GAE) g^{-1} of fresh weight (FW). A standard gallic acid calibration curve (0–250 mg L^{-1}) was used.

2.6.2. Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) was measured using the $AlCl_3$ method, adapted from the procedure reported by Zhishen et al. [71]. The measurement of absorbance at 510 nm after 10 min of incubation was used to quantify TFC, reported as mg quercetin equivalent (QE) g^{-1} of FW. A standard quercetin calibration curve (0–125 mg L^{-1}) was used.

2.6.3. Total Antioxidant Activity

Total Antioxidant Activity (TAA) of white grape and red grape extracts was evaluated via the ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) assay, based on the ability of antioxidants to decrease ABTS pre-formed radicals, according to the procedure of Re et al. [72]. The ABTS+• solution was produced and diluted to an absorbance of 0.7. Each sample was then mixed with the active solution

Fig. 1. White and red grapes at the beginning of the storage (T0) and at the end of treatments with chitosan samples at room temperature (RT) and 4 °C. Treatments: untreated fruits (Ctrl-), solvent, coating with 0.5 and 1 % of commercial chitosan (K chitosan), not decolorized (No Dec) and decolorized (Dec) chitosan from *H. illucens* pupal exuviae.

to inhibit up to 80 % the absorbance of the blank at 734 nm. The treatment lasted 30 min. The TAA quantification was expressed as mg of Trolox equivalent (TE) g^{-1} of FW. A standard Trolox calibration curve (0–125 mg L^{-1}) was used.

2.7. Statistical analysis

All measurements were performed in triplicate and data were expressed as mean ± standard deviation. Data were analyzed by one-way Anova and Tukey's *post-hoc* test. Statistical analyses were performed using a GraphPad Prism version 6.0.0 for Windows (GraphPad Software, San Diego, California USA).

3. Results and discussion

3.1. Chitosan production and chitosan coating solutions preparation

Deacetylation degree (DD) of chitosan from *H. illucens* proved to be similar to that of chitosan from crustaceans, falling in the same interval, between 85 and 90 % [57,68]. Molecular weight (Mw) values, amounting between 30 and 300 kDa, are also within the same range of those identified for crustacean derived chitosan [55]. Viscosity-average molecular weight (M_v) values of 75 kDa for Dec chitosan and 150 kDa for No Dec chitosan, are both below 370 kDa, the M_v of K chitosan [69].

3.2. Evaluation of influence of chitosan coating from H. illucens on grape decay

To investigate the effectiveness of *H. illucens* chitosan coating on white and red grapes, several chemical parameters were evaluated, such as weight loss, TSS, and pH. The experiment was considered completed when most of the berries decayed under both storage conditions tested: for up to 15 days at RT and 50 days at 4 °C, with the red grapes preserving better than the white ones.

From a visual examination, chitosan treatments were effective in preserving the shelf life of the berries in terms of physical decay. Particularly for the white grapes, at RT, the control and solvent showed a clear decay compared to the chitosan-treated berries (Fig. 1). At 4 °C, the low temperature was more effective in preventing decay. However, all chitosan-treated grapes, especially the insect-chitosan, performed better than the untreated control and the solvent alone (Figs. 1–2).

3.2.1. Effect of chitosan coating on weight loss of white and red grapes

Respiration rate and moisture evaporation are important indices of fruit weight loss. The moisture content of fresh products, specifically grapes, is normally above 80 %, so preventing water loss is crucial to improve the shelf life [73]. Chitosan, known for its several beneficial properties, additionally exhibits hygroscopic characteristics enabling the formation of a semi-permeable layer [15,74], limiting water loss and, consequently, weight loss [75,76]. At RT, treatment of white grapes with *H. illucens* chitosan slightly reduced weight loss compared to control and solvent, although there were no statistically significant differences. Particularly, white grapes treated with 0.5 % No Dec chitosan showed the lowest weight loss. Results of all the measurements are reported in Table 1. The weight loss caused by treatment with K chitosan, both at 0.5 % and 1 %, was quite similar to the control and white grapes treated with solvent. At 4 °C, treatment with both K and *H. illucens* chitosan showed significant differences compared to control and to solvent treatments. In particular, for the same tested concentration, *H. illucens* chitosan samples (Dec and No Dec), performed similarly to K

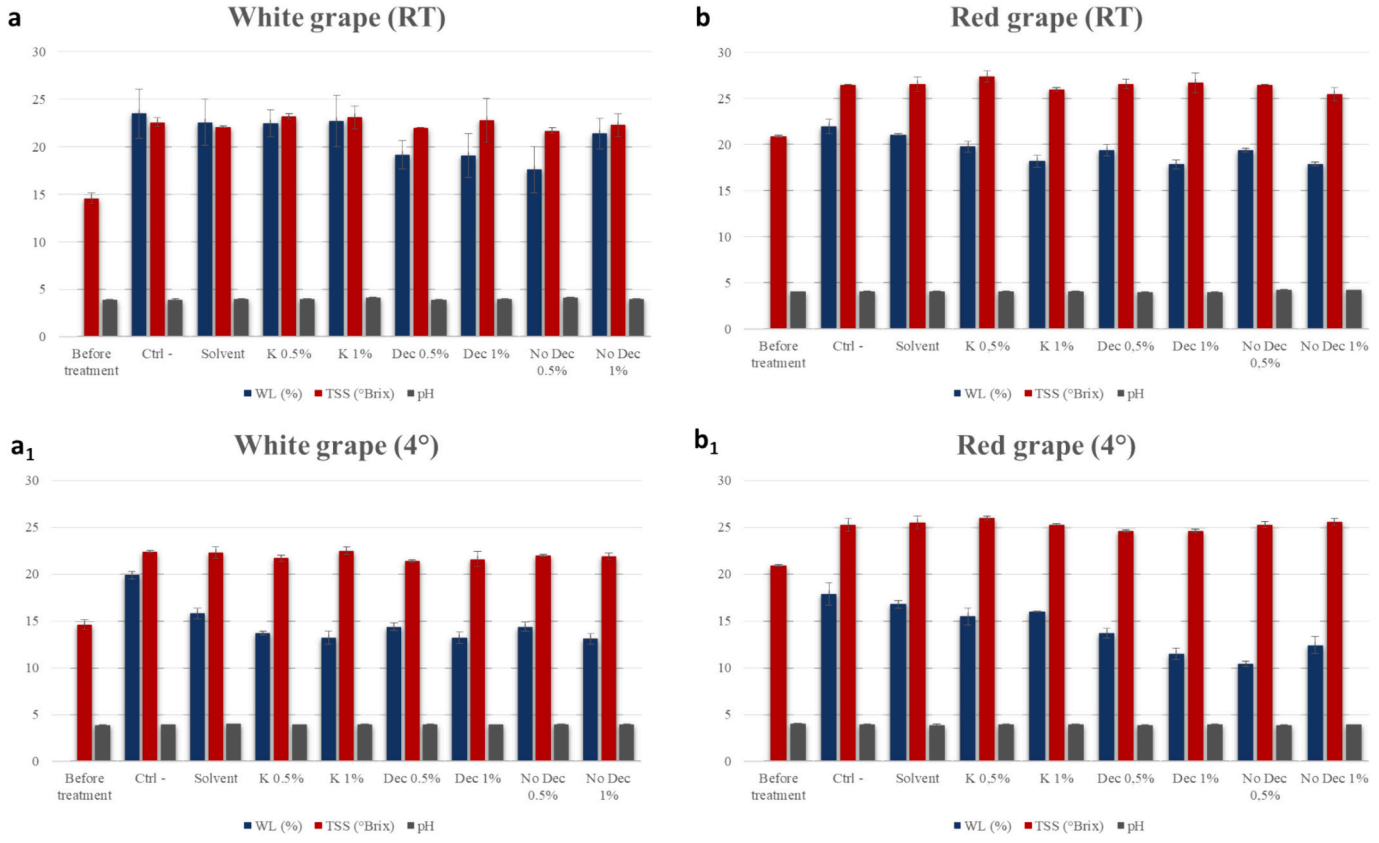


Fig. 2. Graphical representation of the results obtained from the analysis of the chemical-physical parameters (WL, TSS, pH) on white grapes at RT (a) and 4 °C (a_1) and on red grapes at RT (b) and 4 °C (b_1), respectively.
Treatments: untreated fruits (Ctrl-), solvent, coating with 0.5 and 1 % of commercial chitosan (K chitosan), not decolorized (No Dec) and decolorized (Dec) chitosan from *H. illucens* pupal exuviae.

Table 1
Results of evaluation of Weight Loss (WL), Total Soluble Solids (TSS) content, and pH on white and red grapes stored at RT and 4 °C. Treatments: negative control (Ctrl-), solvent, coating with pupal exuviae not decolorized (No Dec), decolorized (Dec) and commercial (K) chitosan, both at 0.5 % and 1 %. Data are reported as mean ± standard deviation of 3 independent replicates. Different letters indicate statistically significant differences among values reported in each column for each experimental condition (RT or 4 °C), analyzed by one-way ANOVA and Tukey *post-hoc* test ($p < 0.05$).

White grape			
Treatments	WL (%)	TSS (°Brix)	pH
Before treatment		14.6 ± 0.56	3.89 ± 0.01
RT			
Ctrl -	23.5 ± 2.6^{a}	22.6 ± 0.5^{a}	3.93 ± 0.09^{bc}
Solvent	22.6 ± 2.4^{a}	22.1 ± 0.1^{a}	3.94 ± 0.06^{bc}
K 0.5 %	22.5 ± 1.4^{a}	23.2 ± 0.3^{a}	3.97 ± 0.07^{abc}
K 1 %	22.7 ± 2.7^{a}	23.1 ± 1.2^{a}	4.10 ± 0.10^{ab}
Dec 0.5 %	19.2 ± 1.5^{a}	22.0 ± 0.0^{a}	3.91 ± 0.00^{c}
Dec 1 %	19.1 ± 2.3^{a}	22.8 ± 2.3^{a}	3.97 ± 0.01^{ab}
No Dec 0.5 %	17.6 ± 2.4^{a}	21.7 ± 0.3^{a}	4.12 ± 0.02^{a}
No Dec 1 %	21.4 ± 1.6^{a}	22.3 ± 1.2^{a}	3.97 ± 0.06^{abc}
4 °C			
Ctrl -	19.9 ± 0.4^{a}	22.4 ± 0.1^{a}	3.93 ± 0.01^{ab}
Solvent	15.8 ± 0.6^{b}	22.3 ± 0.6^{a}	4.00 ± 0.01^{a}
K 0.5 %	13.7 ± 0.2^{c}	21.7 ± 0.3^{a}	3.92 ± 0.02^{b}
K 1 %	13.2 ± 0.7^{c}	22.5 ± 0.4^{a}	3.96 ± 0.04^{ab}
Dec 0.5 %	14.4 ± 0.4^{bc}	21.4 ± 0.1^{a}	3.98 ± 0.00^{ab}
Dec 1 %	13.2 ± 0.6^{c}	21.6 ± 0.8^{a}	3.93 ± 0.00^{b}
No Dec 0.5 %	14.4 ± 0.5^{c}	22.0 ± 0.1^{a}	3.97 ± 0.03^{ab}
No Dec 1 %	13.1 ± 0.6^{c}	21.9 ± 0.4^{a}	3.96 ± 0.04^{ab}

Red grape			
Treatments	WL (%)	TSS (°Brix)	pH
Before treatment		20.9 ± 0.14	4.04 ± 0.03
RT			
Ctrl -	22.0 ± 0.8^{a}	26.5 ± 0.0^{a}	4.11 ± 0.03^{a}
Solvent	21.1 ± 0.1^{ab}	26.6 ± 0.8^{a}	4.08 ± 0.01^{a}
K 0.5 %	19.8 ± 0.6^{bc}	27.4 ± 0.6^{a}	4.08 ± 0.06^{a}
K 1 %	18.2 ± 0.7^{cd}	26 ± 0.2^{a}	4.09 ± 0.04^{a}
Dec 0.5 %	19.4 ± 0.6^{cd}	26.6 ± 0.5^{a}	3.99 ± 0.03^{c}
Dec 1 %	17.9 ± 0.5^{d}	26.7 ± 1.1^{a}	4.00 ± 0.02^{c}
No Dec 0.5 %	19.4 ± 0.2^{d}	26.5 ± 0.0^{a}	4.22 ± 0.06^{a}
No Dec 1 %	17.9 ± 0.2^{d}	25.5 ± 0.7^{b}	4.22 ± 0.01^{b}
4 °C			
Ctrl -	17.9 ± 1.2^{a}	25.3 ± 0.7^{ab}	3.98 ± 0.00^{a}
Solvent	16.8 ± 0.4^{abc}	25.5 ± 0.7^{ab}	3.89 ± 0.07^{a}
K 0.5 %	15.5 ± 0.9^{bc}	26.0 ± 0.2^{a}	3.92 ± 0.04^{a}
K 1 %	16.0 ± 0.04^{ab}	25.3 ± 0.1^{ab}	3.95 ± 0.02^{a}
Dec 0.5 %	13.7 ± 0.5^{cd}	24.6 ± 0.1^{b}	3.88 ± 0.03^{b}
Dec 1 %	11.5 ± 0.6^{de}	24.6 ± 0.2^{b}	3.93 ± 0.04^{a}
No Dec 0.5 %	10.4 ± 0.3^{e}	25.3 ± 0.3^{ab}	3.89 ± 0.02^{a}
No Dec 1 %	12.4 ± 0.9^{e}	25.6 ± 0.4^{ab}	3.93 ± 0.00 ^{a}

chitosan in weight loss.

For red grapes at RT, K chitosan at the highest concentration significantly reduced the weight loss compared to solvent-only treatment.

Treatments with *H. illucens* chitosan, at all tested concentrations, were effective in maintaining weight loss, significantly compared to control and solvent. Particularly, 1 % chitosan samples, compared to 0.5 %, were the most effective in containing the weight loss of red grapes at RT, although not statistically significant. Shiri et al. [32] also studied the effect of K chitosan on grapes at 0.5 % and 1 %, kept at a low temperature, showing that an increase in chitosan concentration is effective for better weight loss containment. In the cold condition (4 °C), No Dec, at both concentrations, and 1 % Dec chitosan were the most effective compared with K chitosan and controls. At 0.5 %, Dec chitosan resulted in a weight loss statistically similar to 0.5 % K chitosan. In general, for both red and white grapes, our results are consistent with those obtained by Sabir et al. [1], who verified a significantly higher weight loss of untreated grapes than those treated with chitosan. This effect is more noticeable at controlled temperature than at RT, as consequence of the refrigeration influence. Based on our data, we can confirm the hypothesis of a barrier effect caused by chitosan [77], which limits water loss and partially reduces dehydration in the grape bunches. The reduction in weight loss is presumably also attributed to the CO_2 and O_2 regulation, resulting from the biopolymer coating [9]. Our results are also comparable to those of Eshghi et al. [13], who treated grapes with chitosan conjugated with another polysaccharide, gum ghatti, and stored them at 4 °C, observing a weight loss of approximately 15 %. In contrast, treatment with chitosan from *H. illucens* was effective in containing weight loss better than K chitosan, for which Hu et al. [78] reported weight reductions of up to 25 %. Hence, the results obtained employing insect-chitosan are very promising. The effectiveness of the biopolymer in containing weight loss has been demonstrated, in several studies, on different fruits [57,69,79,80]. As reported in Triunfo et al. [69,70], the application of *H. illucens* chitosan on fresh fruit, was effective in limiting weight loss, often better compared to the crustaceans derived-biopolymer.

3.2.2. *Effect of chitosan treatment on Total Soluble Solids (TSS) content of white and red grapes*

For both white and red grapes, the Total Soluble Solids (TSS) value increased throughout the storage period of the treated fruit, as also reported in the study of Eshghi et al. [13], on the same fruit.

At RT, for white grapes, there were no statistically significant differences among the treatments with the two controls (Ctrl - and Solvent), although *H. illucens* chitosan slightly contained the increase in TSS. Specifically, Dec samples had a similar behavior, regardless of concentration (Table 1, Fig. 2). As for No Dec chitosan samples, the 0.5 % had the best containing effect on white grapes. Youssef and Roberto [81] reported that the containment and the decrease in the TSS content in grapes are consequences of the advancement of the fruit respiration, linked to the absence of external sources of nutrients. As far as red grapes at RT are considered, no significant differences were detected in the effect obtained on grapes treated with the biopolymer. Specifically, insect-chitosan treatments showed no statistical differences, compared to K chitosan treatments. However, 1 % No Dec chitosan resulted in a better containment effect than the other samples, statistically significant compared to 0.5 % K chitosan. As reported in other studies [32], the high TSS levels achieved in our study may be linked to the reduction of oxygen supply on the grapes, which inhibited the fruit respiration [82]. Also Romanazzi [83] confirmed the effectiveness of applying chitosan as a coating for table grapes to inhibit the respiration rate of the fruit itself.

At 4 °C, on red grapes, *H. illucens* Dec chitosan was effective in maintaining the increase in TSS value, yielding statistically significant results compared to 0.5 % K chitosan. Also in the work of Shiri et al. [32], crustaceans chitosan was efficacious in containing the increase in TSS, although at higher concentrations compared to those used in our work. The results are in line with what was reported in the work of Eshghi et al. [84]. Indeed, a great variation in TSS occurred in fruits where water loss was reduced, as chitosan treatment decreases metabolic activity due to changes in the internal atmosphere of the fruit. Also in other studies, an increase in TSS content has been demonstrated on both treated and untreated fruit, in some cases mainly dependent on the storage time [85]. It can be hypothesized a correlation of these results with the inhibition of fruits respiration, resulting from a reduction of O_2 on its surface [82].

Our treatments, therefore, showed to be effective in containing the increase in TSS for both white and red grapes. This is in accordance with what is also reported in literature [79,86–89], although it was not

possible to make a direct comparison because this is the first study where *H. illucens* chitosan is used on grapes. Our results agree with other studies on insect-chitosan coating for fresh fruit [57,70]. This confirms the enhanced effectiveness of chitosan from *H. illucens* compared to crustacean-derived chitosan. However, further investigations are necessary to understand the correlation between the chitosan concentration and the respiration rate, and thus the action on O_2 and CO_2 levels.

3.2.3. Effect of chitosan treatment on variation of pH of white and red grapes

As the storage period progresses, the acidity of the fruits normally decreases, resulting in an increase in product pH. This is mainly attributable to the conversion of acids and starch into sugars [90]. Therefore, the use of chitosan as coating is proven to be effective in slowing down the fruit acid metabolism [91].

Regarding white grapes conserved at RT, 0.5 % Dec chitosan exhibited a significant restraining effect on pH increase. Indeed, in this case, the effect was statistically significant compared with both controls (Ctrl - and Solvent). However, at 4 °C, the containing effect was less pronounced, although there was evidence that 1 % Dec *H. illucens* chitosan had a statistically significant effect compared to the solvent, similarly to that obtained after treatment with K chitosan at the lowest concentration (Table 1).

On red grapes, Dec chitosan showed remarkable results in containing pH increase, compared to both controls (Ctrl - and Solvent). These results were statistically significant in comparison to K chitosan, at the respective concentrations. The treatment with K chitosan, in our experiments, either almost maintained or even increased the pH value compared to the control, consistent with the findings of Irkin and Guldas [27] for both concentrations. Even at 4 °C, 0.5 % Dec chitosan was particularly effective in containing the increase in pH, significantly more than the control, the solvent, and all the other chitosan samples. However, K chitosan samples (0.5 % and 1 %), 1 % Dec chitosan and both No Dec chitosan sample were also effective in preventing an increase of this parameter, although they were not statistically significant (Table 1).

Fruit pH can also increase due to the presence of fungi and molds, which use organic acids as growth substrate [86]. However, a beneficial effect of handling crustacean-derived chitosan to reduce the alkalization process has been demonstrated, even in other types of fruit [86,91,92].

3.3. Evaluation of chitosan treatment on phenolic compounds and antioxidant activity of white and red grape

Changes in nutraceutical properties were evaluated by quantifying TPC, TFC and TAA of white and red grapes. The obtained results are reported in Table 2 and Fig. 3.

3.3.1. Total Phenolic Content (TPC)

In this study, the application of the different coating treatments induced an increase in the TPC of both white and red grapes, for both storage conditions tested. The degradation of phenolic compounds normally occurs via an enzyme, the polyphenol oxidase, which can be effectively inhibited by chitosan. This ability of the biopolymer to prevent the reduction of phenolic compounds in the fruits can be attributed to this inhibition [93].

The TPC, at harvest, was higher for red grapes than the white ones, around 0.587 mg GAE g^{-1} and 0.185 mg GAE g^{-1}, respectively. Regarding white grapes at RT, all treatments with chitosan led to an increase in TPC compared to both the solvent and the control, although significant differences were not always observed. The 1 % K treatment was more effective than the 0.5 % K. Dec chitosan samples also showed enhanced TPC compared to the control and to the solvent, although they did not exhibit any significant differences. As for No Dec chitosan samples, the sample at the highest concentration led to the greatest increase in TPC, which was statistically significant compared to the control.

Table 2
Results of evaluation of Total Phenols Content (TPC), Total Flavonoid Content (TFC) and Total Antioxidant Activity (TAA) on white and red grapes stored at RT and 4 °C. Treatments: negative control (Ctrl -), solvent, coating with pupal exuviae not decolorized (No Dec), decolorized (Dec) and commercial (K) chitosan, both at 0.5 % and 1 %. Data are reported as mean ± standard deviation of 3 independent replicates. Different letters indicate statistically significant differences among values reported in each column for each experimental condition (RT or 4 °C), analyzed by one-way ANOVA and Tukey *post-hoc* test ($p < 0.05$). GAE, gallic acid equivalents; QUE, quercetin equivalents; TE, Trolox equivalents.

White grape			
Treatments	TPC (mg GAE g^{-1} FW)	TFC (mg QUE g^{-1} FW)	TAA (mg TE g^{-1} FW)
Before treatment	0.145 ± 0.03	0.190 ± 0.005	0.190 ± 0.03
RT			
Ctrl -	0.185 ± 0.04^{b}	0.220 ± 0.03^{c}	0.209 ± 0.02^{d}
Solvent	0.226 ± 0.04^{ab}	0.213 ± 0.02^{c}	0.311 ± 0.02^{b}
K 0.5 %	0.252 ± 0.04^{ab}	0.230 ± 0.02^{bc}	0.295 ± 0.01^{bc}
K 1 %	0.320 ± 0.01^{ab}	0.247 ± 0.02^{abc}	0.309 ± 0.03^{b}
Dec 0.5 %	0.268 ± 0.04^{ab}	0.258 ± 0.02^{abc}	0.255 ± 0.01^{cd}
Dec 1 %	0.301 ± 0.05^{ab}	0.290 ± 0.02^{ab}	0.310 ± 0.01^{b}
No Dec 0.5 %	0.285 ± 0.04^{ab}	0.297 ± 0.03^{a}	0.323 ± 0.01^{b}
No Dec 1 %	0.351 ± 0.06^{a}	0.293 ± 0.02^{ab}	0.387 ± 0.02^{a}
4 °C			
Ctrl -	0.201 ± 0.04^{c}	0.216 ± 0.03^{bc}	0.266 ± 0.01^{bcd}
Solvent	0.212 ± 0.03^{c}	0.203 ± 0.06^{c}	0.212 ± 0.03^{de}
K 0.5 %	0.218 ± 0.03^{bc}	0.220 ± 0.03^{abc}	0.218 ± 0.03^{cde}
K 1 %	0.245 ± 0.04^{abc}	0.250 ± 0.03^{abc}	0.205 ± 0.01^{e}
Dec 0.5 %	0.336 ± 0.05^{ab}	0.273 ± 0.02^{abc}	0.269 ± 0.01^{bc}
Dec 1 %	0.265 ± 0.04^{abc}	0.280 ± 0.02^{ab}	0.363 ± 0.02^{a}
No Dec 0.5 %	0.251 ± 0.04^{abc}	0.297 ± 0.02^{a}	0.269 ± 0.01^{bc}
No Dec 1 %	0.364 ± 0.06^{a}	0.305 ± 0.02^{a}	0.291 ± 0.01^{b}

Red grape			
Treatments	TP (mg GAE g^{-1} FW)	TFC (mg QUE g^{-1} FW)	TAA (mg TE g^{-1} FW)
Before treatment	0.587 ± 0.05	0.200 ± 0.03	0.561 ± 0.004
RT			
Ctrl -	0.721 ± 0.06^{b}	0.270 ± 0.02^{c}	0.876 ± 0.04^{d}
Solvent	0.734 ± 0.06^{b}	0.280 ± 0.02^{bc}	0.885 ± 0.09^{d}
K 0.5 %	0.754 ± 0.07^{b}	0.300 ± 0.03^{abc}	0.937 ± 0.10^{d}
K 1 %	0.783 ± 0.02^{b}	0.308 ± 0.03^{abc}	1.06 ± 0.10^{d}
Dec 0.5 %	0.897 ± 0.07^{ab}	0.330 ± 0.04^{abc}	1.44 ± 0.06^{c}
Dec 1 %	0.885 ± 0.07^{ab}	0.352 ± 0.02^{abc}	1.68 ± 0.03^{b}
No Dec 0.5 %	0.819 ± 0.06^{b}	0.360 ± 0.04^{ab}	1.39 ± 0.07^{c}
No Dec 1 %	1.03 ± 0.07^{a}	0.375 ± 0.03^{a}	1.97 ± 0.04^{a}
4 °C			
Ctrl -	0.678 ± 0.06^{ab}	0.213 ± 0.03^{abc}	0.745 ± 0.06^{c}
Solvent	0.690 ± 0.06^{ab}	0.206 ± 0.06^{c}	0.706 ± 0.08^{c}
K 0.5 %	0.616 ± 0.06^{b}	0.210 ± 0.02^{bc}	0.828 ± 0.07^{c}
K 1 %	0.706 ± 0.06^{ab}	0.200 ± 0.04^{abc}	1.10 ± 0.10^{b}
Dec 0.5 %	0.797 ± 0.06^{a}	0.194 ± 0.04^{abc}	1.50 ± 0.06^{a}
Dec 1 %	0.814 ± 0.06^{a}	0.233 ± 0.04^{ab}	1.22 ± 0.09^{b}
No Dec 0.5 %	0.749 ± 0.06^{ab}	0.207 ± 0.04^{abc}	1.38 ± 0.07^{ab}
No Dec 1 %	0.796 ± 0.06^{a}	0.227 ± 0.04^{a}	1.48 ± 0.06^{a}

At 4 °C, white grapes TPC did not exhibit any major differences compared to the same treatments at RT. Dec chitosan was more effective than K chitosan, at the same concentrations. Particularly, 0.5 % Dec chitosan significantly increased the TPC, also compared to the control and solvent. For No Dec samples, on the other hand, the highest concentration (1 %) was notably effective in increasing the TPC of the treated grapes, as also reported for the same grapes treated at RT.

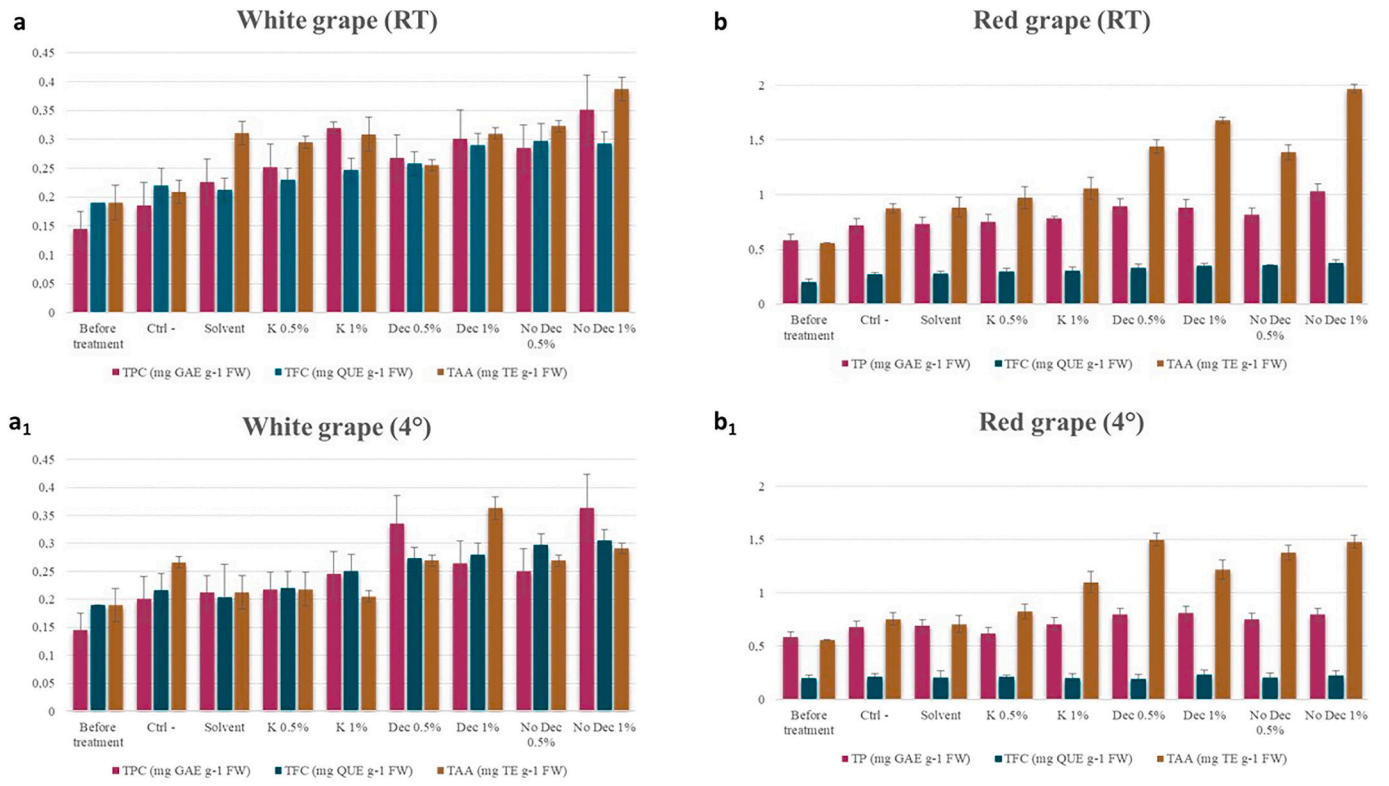


Fig. 3. Graphical representation of the results obtained from the quantification of TPC, TFC and TAA of white grapes at RT (a) and 4 °C (a_1) and on red grapes at RT (b) and 4 °C (b_1), respectively.
Treatments: untreated fruits (Ctrl-), solvent, coating with 0.5 and 1 % of commercial chitosan (K chitosan), not decolorized (No Dec) and decolorized (Dec) chitosan from *H. illucens* pupal exuviae.

An increase in TPC was also observed for red grapes at both RT and 4 °C. For the red grapes treated at RT, K chitosan, both 0.5 % and 1 %, did not cause a significant increase in TPC, compared to the control and solvent. Dec chitosan samples, on the other hand, caused an increase in TPC in the treated grapes, although the difference was not statistically significant compared to the treatments with K chitosan, solvent, and negative control. However, 1 % No Dec chitosan, as observed in white grapes, was the most effective treatment, causing a statistically significant increase in TPC compared to solvent and control. This treatment was also more effective than K chitosan treatment at both concentrations. Regarding the 4 °C treatments, Dec chitosan caused the greatest rise in TPC, together with 1 % No Dec chitosan. 0.5 % No Dec chitosan also resulted in an increase in TPC, indicating that bleaching is not a factor contributing to an improvement in TPC. For both white and red grapes, the obtained results are very encouraging. Indeed, cold storage condition, that normally delays the bleaching of fresh fruit, is not the determining factor in preventing the reduction of the TPC of these fruits. Interestingly, for both types of grapes, RT storage was the most effective, demonstrating an excellent ability of chitosan to preserve the fruit, regardless of the preservative action of cold. The increase in TPC in samples treated with chitosan is generally stronger than untreated ones.

For all treatments and for both grape varieties, the highest concentration of No Dec chitosan was the most effective. This result is consistent with findings reported by Shiri et al. [32]. Meanwhile, Sabir et al. [1] reported an increase in the TPC of the control at the end of the processing period, greater than chitosan treatments at all tested concentrations, resulting worse than our results. In literature, there are several studies describing the positive influence of chitosan treatment on TPC of different fruit types [69,70,84,86,94]. Maintaining and/or increasing TPC is essential for human health, as it not only denotes the antioxidant activity of chitosan, but also has an antibacterial effect on spoilage microorganisms in fresh products [95].

A comparison with literature is not possible either because, to the best of our knowledge, this is the first work on the treatment of grapes with *H. illucens* chitosan. The effectiveness of insect-derived chitosan in stimulating phenolic biosynthesis is inarguable. Actually, the most quoted action mechanism of the biopolymer is related to its influence on the increase of the expression of several genes involved in the phenylpropanoid biosynthetic pathway [96].

3.3.2. *Total Flavonoid Content (TFC)*

The TFC of white and red grapes increased during treatment with insect-chitosan, at both RT and 4 °C. Flavonoids represent a group of physiologically active secondary metabolites [97]. Many plant products contain flavonoids, making these foods of fundamental importance in human diet [98,99]. During storage, white grapes treated at RT with 1 % Dec chitosan showed a significant increase in TFC compared to both control and solvent. The activity was also significant compared to K chitosan, at both 0.5 % and 1 %. This factor is probably related to insect-chitosan molecular weight, which is lower than that of crustaceans, as also reported in literature [57,70]. No Dec chitosan revealed good activity on the processed fruit, also for this parameter; indeed, at both concentrations tested, it showed a significant increase in TFC, compared to both control and solvent. Cold storage, on the contrary, did not seem to have a substantial influence on the spoilage of white grapes. However, for this treatment, the increase was statistically significant for No Dec chitosan, both 0.5 % and 1 %, compared to both control and solvent. On the contrary, no significant differences between the white grapes treated with No Dec chitosan and those treated with Dec and K chitosan were observed.

The data obtained for the treatments on red grapes were consistent with the analyses conducted on white grapes. Indeed, at RT, the greatest increment in TFC of the treated fruit was observed. In this case as well, both 1 % No Dec and Dec chitosan samples led to a significant increase in

TFC. There was also a higher efficacy compared to K chitosan, although the rise was not substantial. As already identified for the analyses carried out on white grapes, low-temperature storage did not have a significant impact on the preservation of the fruit. Thus, only Dec and No Dec chitosan samples at the highest concentration showed a significant increase compared to the solvent. It is noteworthy that the solvent solution had no effect on the shelf life parameters and, therefore, on the increased content of secondary metabolites, as reported in literature on other fruits [57].

The increase in TFC, therefore, appears to be an important parameter regarding the shelf life of grapes, both white and red, which is typically more pronounced at higher concentrations. This aligns with findings reported in a study on "*El-Bayadi*" table grape showing an increase in the TFC of the fruit treated with K chitosan at 1 %, similarly to our results [100].

In some studies, although an increase in TPC is reported, consistently with our work, TFC is stated to decrease [32]. Therefore, the observed increase in TFC is of particular importance, especially considering that this is not linked to cold storage, as observed in other studies on fresh fruit coated with crustacean chitosan [101], where the increase in the content of these metabolites was probably also related to this storage condition. Therefore, *H. illucens* chitosan has proven to be effective in increasing the shelf life of both white and red grapes, thus enhancing their nutraceutical properties.

3.3.3. Total Antioxidant Activity (TAA)

TPC and TFC are closely linked to antioxidant activity, and they are used as indicators of the preservation state of fruits. Chitosan exhibits excellent antioxidant qualities, helping to extend the shelf life of processed fresh products [70,86,102]. Indeed, Petriccione et al. [101] reported that chitosan enhances the activity of several antioxidant enzymes, thus protecting fruit cells from oxidative damage [13]; this rise is in line with that obtained for flavonoids in this same study. Antioxidant activity of both white and red grapes processed with insect-chitosan coatings increased, at both storage conditions, compared to the control, similarly with what has already been reported in other studies on chitosan-based coating obtained from crustaceans. Al Qurashi et al. [100] assumed that chitosan treatment enhanced enzymatic and non-enzymatic antioxidant systems of the coated fruit, resulting in a higher TAA value than in the control, probably due to the effect of the biopolymer on free radicals. It has been reported that preservation could be related to the synergic activity of the application of chitosan with the cold condition [1], whereas in our study the increase of shelf life was also evident at RT; indeed, for white grapes, the increase in TAA was even greater at RT than at 4 °C. This finding is particularly interesting since it suggests that the preservation of shelf life of grapes is due to the biopolymer itself and not to the influence of external storage conditions. Concerning the white grapes at RT, the fruit treated with No Dec chitosan at the highest concentration (1 %) showed the best preservation rate and, consequently, the greatest increase in TAA (0.387 mg TE g^{-1}), which was statistically significant, both compared to the control (0.209 mg TE g^{-1}) and to the solvent (0.311 mg TE g^{-1}). Even at the lowest concentration, No Dec sample showed an increase in TAA, that was statistically significant compared to the control. Both No Dec samples, as well as Dec chitosan at 1 % concentration, caused higher TAA increase than K chitosan. Also at 4 °C, the highest concentration of Dec chitosan had the best effect, which differed statistically from the Ctrl-, from the solvent and also from all other chitosan samples, both from crustaceans and insects. Both Dec and No Dec chitosan samples at 0.5 % had a positive effect, when compared to the solvent but not to the control.

In red grapes, the starting antioxidant activity was higher than that in white grapes (0.561 mg TE g^{-1} vs 0.209 mg TE g^{-1}). At RT, *H. illucens* chitosan samples, both Dec and No Dec, resulted in a statistically significant increase in TAA, compared to the Ctrl- (0.876 mg TE g^{-1}), to the solvent (0.885 mg TE g^{-1}), and to K chitosan (0.937–1.06 mg TE g^{-1}). Once again, the highest concentration of *H. illucens* chitosan was the most effective, with the greatest increase occurring with No Dec chitosan (1.97 mg TE g^{-1}). At 4 °C, similarly, all insect-chitosan samples have statistically significant activity in comparison to the control and to the solvent alone, with the greatest activity again occurring with 1 % No Dec and with 0.5 % Dec chitosan. In other studies where chitosan derived from crustaceans was utilized, only the highest concentration led to an increase of the antioxidant activity [32], while lower concentrations resulted in a decrease compared to the control. Therefore, it seems that insect-chitosan performs better than crustacean one, even at lower concentrations. The effectiveness of chitosan from *H. illucens* in enhancing the antioxidant activity of treated fruit is not influenced by temperature variation; rather, the only discriminating factor is the source of the biopolymer itself. Our work confirms the viability of using pupal exuviae chitosan as a coating for the preservation of white and red grapes, particularly due to its ability to increase the TAA of the fruit itself. In some instances, the activity of insect-chitosan was also better than that of K chitosan, as already pointed out in our previous works [57,70].

4. Conclusions

Grapes are fruits highly susceptible to spoilage, primarily due to handling processes during harvest and transport. The adoption of environmentally friendly packaging is gaining importance, not only to minimise environmental impact, but also to reduce costs and food waste.

Our work represents the first study on *H. illucens* chitosan exploitation to extend the shelf life of both white and red grapes. Chitosan from pupal exuviae effectively slowed down the deterioration of the fruit from a chemical-physical perspective, controlling the fluctuation of some critical parameters such as weight loss, TSS and pH.

Additionally, the biopolymer promoted the improvement of TPC and TFC throughout the treatment period. This is also related to an enhancement of antioxidant properties, crucial for human health as they neutralize free radicals, molecules that damage cells and promote aging. Particularly, No Dec chitosan treatment demonstrated the most significant impact on nutraceutical and phenolic compounds, as well as antioxidant activity, for both conditions studied in white grapes. At RT, treatment with No Dec biopolymer also improved TPC, TFC and TAA in red grapes. Conversely, on red grapes stored at 4 °C, these parameters increased with the Dec chitosan sample. Therefore, treatment with *H. illucens* chitosan has proven to be effective in slowing down the decay of white and red grapes, offering to the consumers the possibility to store them for a longer period, with consequent health benefits. Our study constitutes an important element validating the use of insect biopolymers in the agri-food industry for fresh food preservation, thereby reducing food waste.

Funding

This work was supported by University of Basilicata and INPS within PhD Program Industry 4.0. and by Basilicata Region within the framework of FSC "Fondo per lo Sviluppo e la Coesione" (Gestione del ciclo di scarti e sottoprodotti della filiera agroalimentare attraverso la loro bioconversione in prodotti di valore – D.G.R. n. 652 30/09/2022).

CRediT authorship contribution statement

Anna Guarnieri: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Micaela Triunfo:** Writing – review & editing, Methodology. **Dolores Ianniciello:** Writing – review & editing, Methodology. **Francesco Tedesco:** Writing – review & editing. **Rosanna Salvia:** Writing – review & editing, Data curation. **Carmen Scieuzo:** Writing – review & editing, Writing – original draft, Supervision, Data curation. **Eric Schmitt:** Writing – review & editing. **Angela Capece:** Writing – review & editing. **Patrizia Falabella:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data

curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Eric Schmitt reports a relationship with Protix that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

We thank APOFRUIT Italia soc. coop. agricola—Scanzano Jonico, (Matera, Italy) for supporting our work. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

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