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RESEARCH

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Usage of chitosan from *Hermetia illucens* as a preservative for fresh Prunus species fruits: a preliminary analysis

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Abstract

Background Fruit and vegetables are highly perishable. In an era where reducing food waste is absolutely essential, packaging is important for maintaining the postharvest quality of these fresh products. Research is working to reduce the use of synthetic materials, not safe for the environment and human health. In this perspective, chitosan emerges as a viable solution for this purpose, as it is biodegradable, biocompatible and also safe for food application. The growing interest in using insects as a source of chitin has allowed for increased exploitation of insect-based waste products to recover valuable materials, such as biopolymers. The black soldier fly (*Hermetia illucens* L.) is the most widely reared species in Europe for feed production and waste management.

Results In this work, fresh mature apricots (*Prunus armeniaca* L.), nectarines (*Prunus persica* vulgaris Mill.) and yellow peaches (*Prunus persica* var. laevis Gray) were coated with 0.5% and 1% chitosan from the pupal exuviae of *Hermetia illucens*, applied by spraying and stored at room temperature or 4 °C until they decay. Then, to validate the effectiveness of chitosan as a polymer for fruit preservation, several parameters including pH, TSS and weight loss were evaluated.

Conclusions The results showed that chitosan derived from the black soldier fly is as effective as or better than the commercially available crustacean chitosan in maintaining more stable some storage parameters in fresh apricots, nectarines and peaches. Thus, insects, especially *Hermetia illucens*, are confirmed as a viable alternative source of the polymer.

Keywords Black soldier fly, Chitosan coating, Apricots, Nectarines, Peaches

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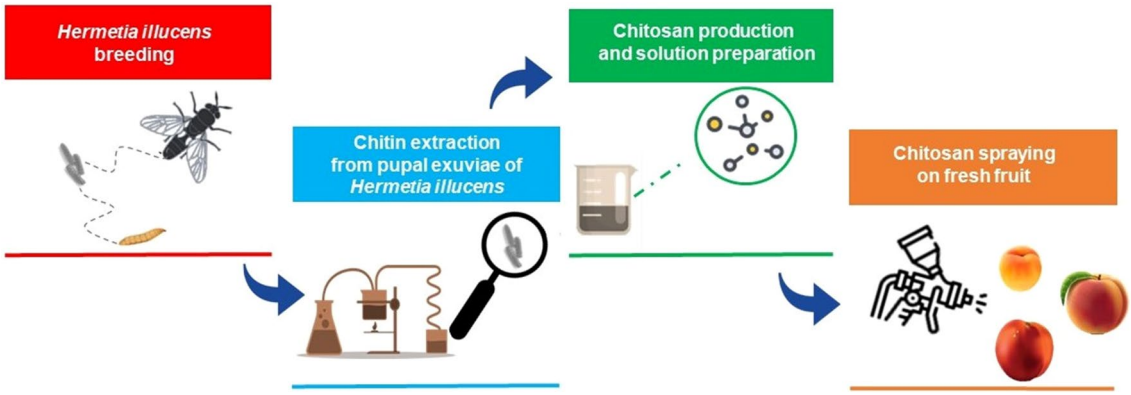
Graphical Abstract



Background

Fruit and vegetables are highly perishable commodities that undergo postharvest deterioration due to weight loss, physiological disorders, and diseases [1–3]. Different packaging methodologies, including edible coating, are relevant for maintaining the postharvest quality of these fresh products. Synthetic polymers, traditionally used for food packaging, have serious problems related to their environmental impact as non-biodegradable materials, despite their good effectiveness as a protective barrier [4]. In 2020, the global value of the packaged food market was estimated at $1.9 trillion, with an expected annual growth rate of 5% [5]. Therefore, research is making increasing efforts to reduce the environmental impact of conventional packaging developing edible preservative coatings based on natural, biodegradable polymers (e.g., proteins, lipids, polysaccharides). Chitosan is one of the most promising biopolymers for this application [6]. It is a linear cationic polysaccharide derived from the deacetylation of chitin, the main structural component of arthropods exoskeleton [7]. Chitosan-based coatings have already demonstrated good efficacy in enhancing the shelf-life of a variety of fresh fruits and vegetables, including strawberries [8], plums [9], pears [10], mangoes [11], berries [12, 13], tomatoes [14], carrots [15], cucumbers [16], bell peppers [17], mushrooms [18], aubergines [19], and also commodities of animal origin, such as meat [20, 21], fish fillets [22, 23] and eggs [24]. Chitosan coatings act as a barrier able to reduce dehydration and weight loss of fresh fruit and vegetables, also delaying maturation and senescence of these products during storage. Chitosan also shows antioxidant and antimicrobial activities [25], thus retarding microbial spoilage and growth of fungal populations [26]. Chitosan has a broad variety of uses due to its distinctive features, versatility, and helpful qualities. It is able to form films and chelate metal ions [27, 28]. Chitosan is also suitable for cosmetic applications [29, 30]. Furthermore, it can be used in the treatment of burns and wounds and as a drug delivery system [31]. Due to its low toxicity, chitosan is even used in the food industry as a natural preservative. It can inhibit the growth of some bacteria, fungi, and yeasts, thus extending the shelf life of food products [32]. In agriculture, chitosan is employed as a biopesticide and a stimulant of plant development [33, 34].

Chitosan is traditionally produced by alkaline hydrolysis of chitin extracted from fishery waste, mainly crustacean shells [35]. Recently, a growing interest in using insects as a source of chitin and chitosan has developed, driven by the numerous large-scale insect breeding facilities that have arisen in many countries. Waste materials from these farms, mainly pupal exuviae generated by the molting of insects from one developmental stage to another, represent a chitin-rich biomass that can be exploited [36, 37]. The black soldier fly (*Hermetia illucens* L.) (BSF) is the most widely reared species in Europe for feed production and waste management [38]. Indeed, BSF larvae, attracted by specific organic volatile compounds [39], can fed on a variety of organic substrates, resulting in a body mass with a high protein and lipid content that can be used for the production of animal feed, biofuels, in cosmetic and biomedical industry [40–51]. The pupal exuviae released by BSF during the transition from the pupal to the adult stage is one of the main waste products of its breeding and contains up to 25% chitin that can be purified to produce chitosan [52].

The aim of this preliminary investigation was to evaluate, for the first time, the ability of chitosan produced from an insect biomass (BSF pupal exuviae) to perform as a protective coating for the preservation of three fresh

fruits (apricots, nectarines and peaches). The purpose of the work was to test its suitability to be used as an alternative to the commercially available chitosan to validate the valorisation of a *H. illucens* breeding waste for an application in the agri-food sector. Obtaining a biopolymer so sought-after on the market, and so useful in the agri-food sector, from an alternative and widely available source, is particularly relevant. Producing chitosan from *H. illucens* not only satisfies the commercial demand for the product but also makes it possible to counteract problems related to the processing of the commercial source, i.e., crustaceans. Indeed, the exploitation of the ocean depths is causing a major environmental problem, prospectively very damaging for the planet. However, the use of this alternative source makes it possible to obtain a biopolymer that provides the same characteristics of the crustacean one, but with a zero ecological footprint. The effect of commercial chitosan from crustaceans used in the preservation of fresh fruit has already been widely studied. In contrast, there are few studies evaluating the effect of insect chitosan. This is the first study evaluating the effect of chitosan derived from *H. illucens* in improving the shelf life of peaches, apricots, and nectarines. Comparing its effect with commercial chitosan, insect chitosan proved to be a viable alternative to the use of crustacean chitosan. The novelty of the work also lies in the utilization of one of the few waste products generated by the breeding of *H. illucens*. We would not only report the production of chitosan and its characterisation [53], but also its application in a valuable economic sector.

Methods

Chitin and chitosan preparation

BSF pupal exuviae were provided by Xflies s.r.l (Potenza, Italy). Larvae were reared on a standard Gainesville diet (30% alfalfa, 50% wheat bran, 20% corn meal), supplied by the animal feed factory Mangimi Losasso s.r.l.—Balvano (Potenza, Italy) [54]. Chitin was extracted from BSF pupal exuviae, both bleached (Bl) and unbleached (Unbl), and heterogeneously deacetylated, as reported in Triunfo et al. [53]. Two heterogeneous chitosan samples were obtained from pupal exuviae chitin: unbleached and bleached chitosan. Commercial crustacean-derived chitin and chitosan, used as benchmark, as well as all reagents were purchased from Merck Millipore (Burlington, MA, USA).

Chitin and chitosan characterization

Characterization of BSF chitin and chitosan was determined using FTIR Jasco 460Plus and scanned in the range of wavelength from 4000 to 500 cm^{-1}. Chitin and chitosan functional groups were defined from the fingerprint of each compound.

For each BSF chitosan samples, the deacetylation degree (DD) by potentiometric titration, according to Jiang et al. method [55], and the viscosity-average molecular weight (Mv) by intrinsic viscosity (η), following the Singh et al. method [56], were determined.

BSF chitosan film-forming ability

The film-forming ability of BSF chitosan was also assessed. The 1% (*w*/*v*) chitosan was dissolved in a 1% (*v*/*v*) aqueous solution of acetic acid, stirred until complete solubilization of chitosan and poured into a Petri dish. The film-forming solution was allowed to dry at room temperature for 72 h, and then films were removed from the Petri dishes [54].

Fruits

Fresh mature apricots (*Prunus armeniaca* L.) and two varieties of the persica species, such as yellow nectarines (*Prunus persica vulgaris* Mill.) with smooth skin and crunchy pulp, and yellow peaches (*Prunus persica var. laevis* Gray), i.e., glabrous fruits with spongy pulp, were supplied by a local agricultural cooperative (APOFRUIT Italia soc. coop. agricola, Scanzano Jonico, Matera, Italy). Fruits without signs of mechanical damage or spoilage, and of similar size, were selected for the experiments.

Preparation and application of coating solutions

Coating solutions were prepared according to the method by Hassan et al. and Tafi et al. [8, 57]. Chitosan (0.5% *w*/*v* or 1% *w*/*v*) was dissolved in a solvent solution consisting of 1% *v*/*v* acetic acid, 2% *v*/*v* glycerol and 0.2% *v*/*v* Tween-80. Two control conditions were set up: untreated fruits (negative control) and treatment with the chitosan-free coating solution (solvent alone). A commercial chitosan (Comm CS) sample was also tested. Fruits were coated by spraying using an aerograph (Martellato s.r.l., Rovigo, Italy) and then dried at room temperature for 30 min. The spraying with the coating solution was repeated twice to ensure uniform surface coverage, as reported by Tafi et al. [57]. One half of the fruit of each treatment was stored at room temperature (RT), while the other half was stored at refrigeration temperature (4 °C). For the evaluation, the fruits were carried to decay.

Weight loss determination

All fruits were weighed every 3 days, using an electronic weighing balance (Sartorius- BCE ENTRIS II, Göttingen, Germany). The weight loss was calculated as the percentage ratio between the weight difference at the beginning (T0) and at the end (Tf) of the storage period, and the initial weight of the fruit:

$$Weight\ loss\ (\%) = \frac{weight\ (g)\ at\ T_0 - weight\ (g)\ at\ T_f}{weight\ (g)\ at\ T_0} \times 100. \quad (1)$$

Measurement of total soluble solids (TSS) and pH

Fruit pulp was homogenized using a laboratory blender and then diluted, suspending 5 g of pulp in 25 ml of distilled water. TSS was determined using a hand refractometer, according to the standard method EN ISO 2173:2003 [58] and expressed as Brix°. The pH of the fruit pulp was measured at RT with a pH meter (Orion Research Inc., Boston, USA). TSS and pH were measured at the beginning and the end of the experiment and the variation of these parameters during the storage period was evaluated.

Statistical analysis

All measurements were performed in triplicate and data were expressed as average ± standard deviation. Data were analyzed by one-way Anova and Bonferroni *post-hoc* test. Statistical analyses were performed using a GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, California USA).

Results

Chitin and chitosan characterization

Spectra of chitin and chitosan obtained by FTIR are provided in Fig. 1a, b. All samples were structurally similar to the commercial polymers. The α form was identified for chitin by observing the split of the amide I band into two peaks at 1650 and 1620 cm^{-1} [59]. Chitosan spectra presented the characteristic bands at 1650 cm^{-1} (amide I) and 1590 cm^{-1} (amide II) [60], confirming its formation after chitin deacetylation. In accordance with Kumirska et al. [59], the higher intensity of the band at 1650 cm^{-1} (amide I) than the one at 1590 cm^{-1} (NH_2 bending) is a qualitative indicator of a lower deacetylation of the chitosan obtained from BSF in comparison with the commercial sample. Chemical characterization of chitosan is reported in Table 1. Chitosan from BSF pupal exuviae features a similar deacetylation degree compared to crustacean-derived chitosan, but with a much lower molecular weight, as reported in Table 1. Both chitosan samples produced from BSF form a uniform and homogeneous film, in terms of surface integrity, thickness, and transparency (Fig. 2). Films were strong enough to be handled without breaking.

Evaluation of the influence of BSF chitosan coating on fruit decay

To evaluate the effectiveness of the chitosan coating from BSF on the *Prunus* species tested, several parameters were analyzed, such as weight loss, TSS content, and pH. The test was considered completed when the fruits reached decay at both storage conditions tested: approximately 8–15 days at RT and 15–26 days at 4 °C. In the present work, a crucial effect of the chitosan coating in inhibiting spontaneous mold growth on apricots, nectarines, and peaches was demonstrated (Fig. 3a, b). Performing a visual evaluation, chitosan treatments were effective in maintaining the shelf life of all fruits, in terms of physical deterioration. Particularly, at RT and for the same period, the control and solvent showed a visible deterioration compared to the BSF chitosan-treated fruits (Fig. 3a). Therefore, the introduction of an appropriate solvent-only control allowed one to recognize whether the effect was attributable to the active polymer alone or to other components of the solution used. In the current instance, since the solvent-only control always gave a higher presence of mold than the other treatments, the effect observed in reducing the incidence of moldy fruits can be attributed to the chitosan itself. In storage at 4 °C, the low temperature has a crucial influence in preventing decay. However, even in this case, all fruits treated with chitosan, particularly BSF chitosan, were better than the untreated control and the solvent alone (Fig. 3b).

Weight loss

In apricots stored at RT, a similar weight loss was observed for all treatments (Table 2). Although not statistically significant compared to the others, the lowest loss was detected in the fruits coated with bleached chitosan. At 4 °C, a significant reduction in the weight loss was observed in apricots treated with both chitosan samples from the BSF pupal exuviae, particularly with 0.5% bleached chitosan and with the commercial one, compared to the negative control. Nevertheless, the solvent-alone coating had a similar but slightly worse effect to the chitosan treatments.

In nectarines, no treatment had a different effect than the negative control, neither at RT nor at 4 °C (Table 3). Although not statistically significant compared to the others, the lowest loss was found in fruits coated with bleached chitosan at both storage conditions.

In peaches, treatment with both samples of chitosan from BSF pupal exuviae was effective in significantly reducing weight loss compared to both the negative control and solvent-coated fruit, regardless of storage temperature (Table 4). In addition, chitosan from BSF also showed a significant effect compared to commercial chitosan, particularly with 0.5% bleached sample, at both storage conditions.

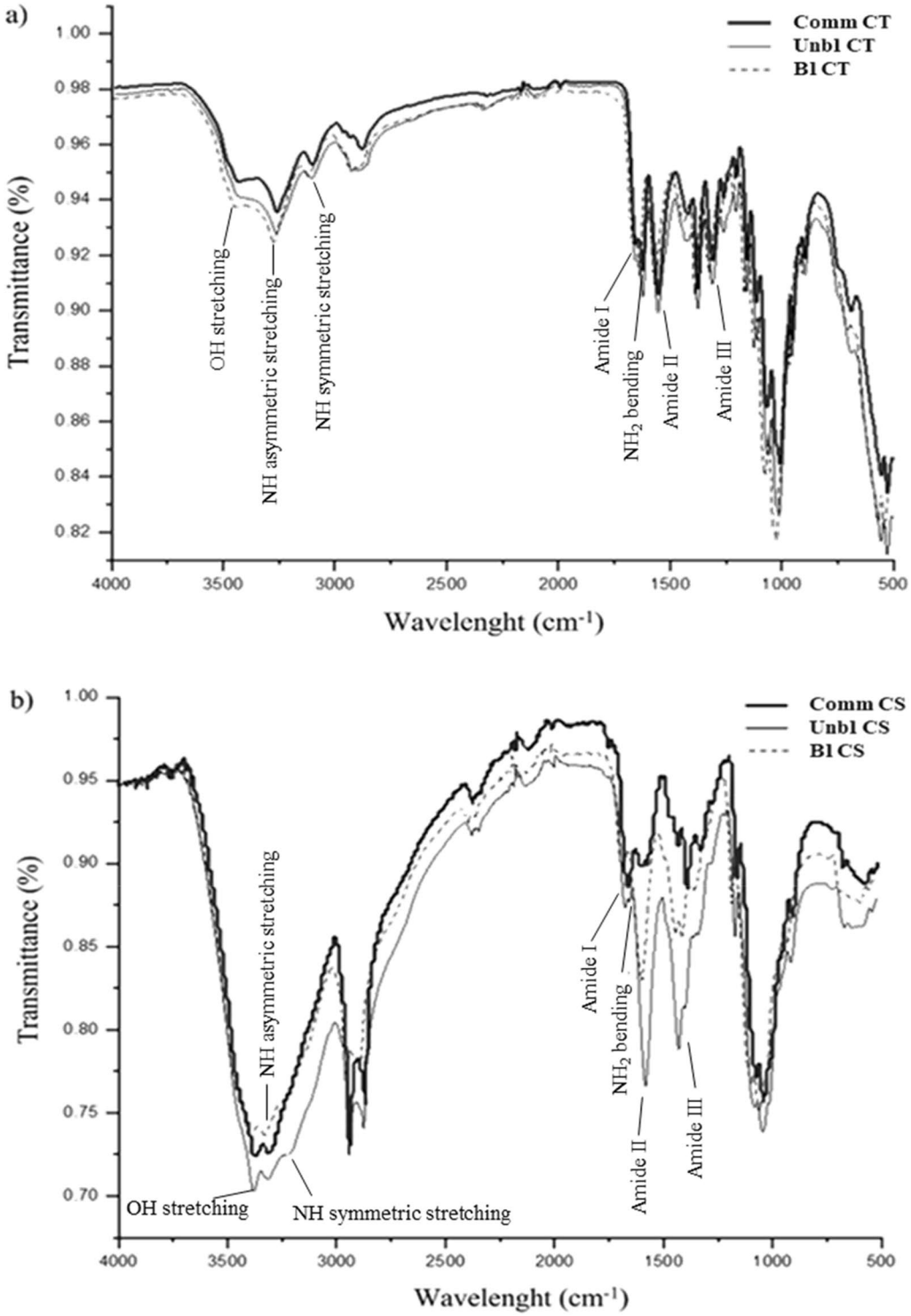


Fig. 1 FTIR spectra of chitin (CT) (**a**) and chitosan (CS) (**b**) produced from BSF pupal exuviae, both unbleached (Unbl CS) and bleached (Bl CS), in comparison with the commercial polymers (Comm CT and Comm CS)

Table 1 Deacetylation degree (DD) and viscosity-average molecular weight (Mv) of chitosan (CS) samples from BSF obtained from both Unbl and Bl CT, and the commercial chitosan (Comm CS)

	DD (%)	M_v (kDa)
Unbl CS	86 ± 2.0	137 ± 3.5[b]
Bl CS	87 ± 1.8	75 ± 3.8[c]
Comm CS	90 ± 1.2	363 ± 4.2[a]

Data are expressed as mean ± standard deviation. Different letters (a,b,c) in the column indicate significant differences ($p < 0.001$) among chitosan samples (data analysed with one-way ANOVA and Bonferroni *post-hoc* test)

Variation of TSS content

TSS content of all fruits increased during storage, at both temperatures.

In apricots stored at RT, the greatest TSS increase occurred in the negative control (Table 2). Fruits treated with solvent alone, commercial and unbleached chitosan from pupal exuviae, had a slightly smaller increase in TSS than the negative control, although statistically similar. Only coating with 0.5% bleached chitosan gave a significant reduction in the TSS increase compared to the negative control. Although no significant differences were detected among treatments on apricots stored at 4 °C (Table 2), the bleached chitosan coating, particularly the 0.5% chitosan sample, proved to be the most powerful in limiting the TSS increase during storage.

In nectarines stored at RT, BSF chitosan (particularly 0.5% Unbl CS and 0.5% Bl CS), and commercial chitosan (particularly coating with 1% Comm CS) were effective in significantly reducing the TSS variation compared to both the negative control and the solvent control (Table 3). At 4 °C storage, although no significant differences were observed among treatments, the bleached chitosan and only 1% unbleached chitosan from BSF showed the lowest TSS variation.

In peaches stored at RT, the highest increase in TSS occurred in negative control which was statistically different from both commercial and BSF chitosan treatments (Table 4). Compared to the commercial chitosan coating, the unbleached chitosan from BSF was more effective in reducing the TSS increase than the bleached one at both concentrations tested. No significant differences were detected among treatments on peaches stored at 4 °C. However, the lowest variation was observed in fruits coated with 0.5% unbleached chitosan from BSF.

Variation of pH

pH of apricots increased during storage, at both temperatures. At RT, pH of apricots coated with all chitosan samples from pupal exuviae and with 1% commercial chitosan increased significantly less than that of fruits belonging to both negative and solvent control (Table 2). Bleached chitosan from BSF had a better effect than commercial one. At 4 °C, all treatments, including the solvent alone, gave a significantly smaller increase in pH than the negative control, to a similar extent.

In nectarines kept at RT, the greatest pH increase was observed in the solvent control (Table 3). All chitosan treatments gave a smaller pH increase than the solvent alone, but only the pupal exuviae chitosan treatments (except 0.5% unbleached chitosan) significantly reduced the pH variation compared to the negative control. All pupal exuviae chitosan coatings had a better effect compared to the commercial one. All treatments with chitosan from pupal exuviae had a better effect than the commercial one. At 4 °C, all treatments reduced the pH variation compared to the negative control and the solvent-alone control. Chitosan from BSF proved to be more effective than the commercial sample. In particular, among pupal exuviae chitosan treatments, the 1% unbleached chitosan and the 0.5% bleached chitosan were the most effective in maintaining the pH of nectarines stable during cold storage.

In peaches, none of the applied treatment significantly reduced the pH increase compared to the negative control (Table 4). Although not statistically significant,

Fig. 2 Chitosan films obtained from BSF pupal exuviae: unbleached (**a**) and bleached (**b**), and commercial chitosan derived from crustaceans (**c**)

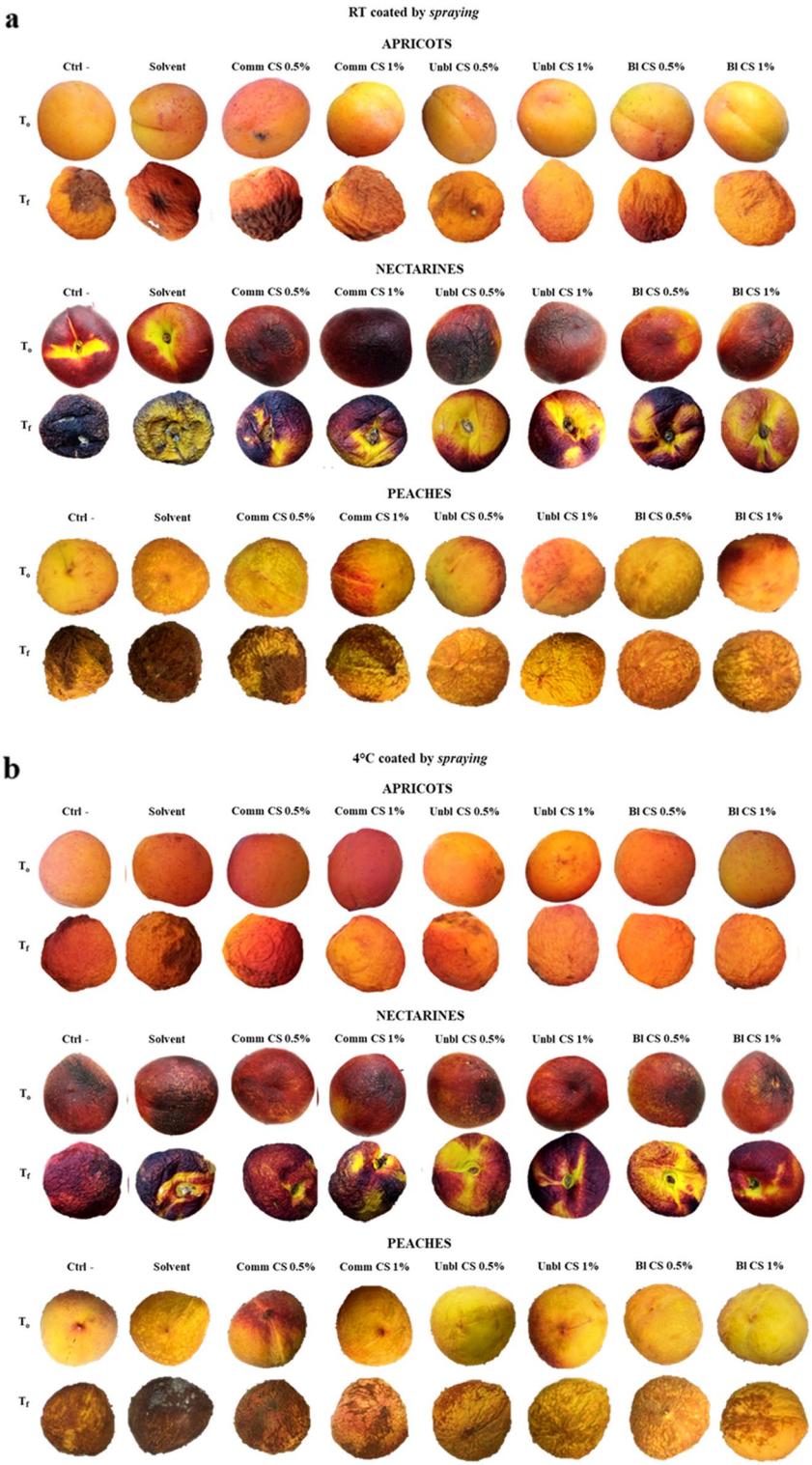


Fig. 3 Pictures of apricots, yellow nectarines and peaches coated by spraying at the beginning (T0) and at the end (Tf) of their storage period at (**a**) RT and (**b**) 4 °C. Treatments: untreated fruits (Ctrl-), solvent, coating with 0.5% and 1% of commercial chitosan (Comm CS), unbleached (Unbl CS) and bleached (Bl CS), chitosan from *H. illucens* pupal exuviae

Table 2 Results of evaluation of weight loss, TSS content and pH of apricots after 14 days of storage at RT and 26 days at 4 °C

Apricots			
Treatments	**Weight loss (%)**	**TSS (°Brix)**	**pH**
Before treatment		2.6 ± 0.2	2.81 ± 0.01
After storage at RT			
Ctrl -	54 ± 3.1^{a}	4.1 ± 0.1^{a}	3.26 ± 0.02^{a}
Solvent	55 ± 3.1^{a}	3.6 ± 0.3^{ab}	3.20 ± 0.01^{b}
Comm CS 0.5%	53 ± 3.4^{a}	3.9 ± 0.1^{ab}	3.17 ± 0.01^{b}
Comm CS 1%	53 ± 3.3^{a}	3.6 ± 0.2^{ab}	3.15 ± 0.02^{bcd}
Unbl CS 0.5%	54 ± 2.9^{a}	3.9 ± 0.1^{ab}	3.16 ± 0.02^{bc}
Unbl CS 1%	53 ± 4.0^{a}	3.6 ± 0.4^{ab}	3.11 ± 0.02^{cd}
Bl CS 0.5%	50 ± 3.1^{a}	3.2 ± 0.2^{b}	3.10 ± 0.01^{d}
Bl CS 1%	50 ± 2.3^{a}	3.8 ± 0.3^{ab}	3.09 ± 0.02^{d}
After storage at 4 °C			
Ctrl -	64 ± 3.0^{a}	5.1 ± 0.1^{a}	3.15 ± 0.02^{a}
Solvent	62 ± 3.3^{ab}	4.8 ± 0.2^{a}	3.02 ± 0.01^{b}
Comm CS 0.5%	60 ± 3.3^{ab}	5.1 ± 0.1^{a}	2.99 ± 0.01^{b}
Comm CS 1%	57 ± 3.4^{ab}	4.9 ± 0.3^{a}	3.00 ± 0.01^{b}
Unbl CS 0.5%	57 ± 2.7^{ab}	4.9 ± 0.1^{a}	3.00 ± 0.01^{b}
Unbl CS 1%	57 ± 3.0^{ab}	5.1 ± 0.3^{a}	3.01 ± 0.02^{b}
Bl CS 0.5%	55 ± 1.9^{b}	4.3 ± 0.2^{a}	3.01 ± 0.01^{b}
Bl CS 1%	57 ± 2.1^{ab}	4.7 ± 0.4^{a}	3.00 ± 0.01^{b}

Treatments: untreated fruits (Ctrl -), solvent only, coating with pupal exuviae unbleached (Unbl CS), bleached (Bl CS) and commercial (Comm CS) chitosan. Means followed by different letters (a,b,c,d) in the column, for each storage condition, are significantly different ($p < 0.05$) by one-way ANOVA and Bonferroni post-hoc test. Each trial contained three triplicates of apricots per each treatment

Table 3 Results of evaluation of weight loss, TSS content and pH of nectarines after 10 days of storage at RT and 21 days at 4 °C

Nectarines			
Treatments	**Weight loss (%)**	**TSS (°Brix)**	**pH**
Before treatment		14.6 ± 0.4	3.93 ± 0.01
After storage at RT			
Ctrl -	53 ± 2.3^{a}	16.9 ± 0.1^{a}	4.57 ± 0.01^{e}
Solvent	53 ± 3.1^{a}	17.1 ± 0.1^{a}	5.00 ± 0.01^{a}
Comm CS 0.5%	51 ± 3.6^{a}	16.1 ± 0.1^{bc}	4.69 ± 0.01^{c}
Comm CS 1%	54 ± 3.3^{a}	15.8 ± 0.2^{c}	4.78 ± 0.01^{b}
Unbl CS 0.5%	54 ± 1.8^{a}	15.7 ± 0.2^{c}	4.64 ± 0.01^{d}
Unbl CS 1%	58 ± 4.4^{a}	15.9 ± 0.1^{bc}	4.22 ± 0.01^{h}
Bl CS 0.5%	52 ± 3.4^{a}	15.7 ± 0.2^{c}	4.53 ± 0.01^{f}
Bl CS 1%	50 ± 3.7^{a}	16.3 ± 0.1^{b}	4.47 ± 0.01^{g}
After storage at 4 °C			
Ctrl -	50 ± 1.5^{a}	24.5 ± 0.8^{a}	4.34 ± 0.01^{a}
Solvent	46 ± 3.8^{a}	23.7 ± 1.3^{a}	4.33 ± 0.01^{a}
Comm CS 0.5%	52 ± 2.6^{a}	26.5 ± 2.1^{a}	4.19 ± 0.01^{b}
Comm CS 1%	52 ± 3.1^{a}	24.1 ± 1.9^{a}	4.21 ± 0.02^{b}
Unbl CS 0.5%	50 ± 3.8^{a}	26.9 ± 2.0^{a}	4.09 ± 0.01^{c}
Unbl CS 1%	51 ± 4.5^{a}	23.7 ± 1.3^{a}	4.03 ± 0.02^{d}
Bl CS 0.5%	48 ± 3.7^{a}	22.9 ± 0.7^{a}	4.02 ± 0.02^{d}
Bl CS 1%	49 ± 3.1^{a}	23.7 ± 1.3^{a}	4.09 ± 0.02^{c}

Treatments: untreated fruits (Ctrl -), solvent only, coating with pupal exuviae unbleached (Unbl CS), bleached (Bl CS) and commercial (Comm CS) chitosan. Means followed by different letters (a,b,c,d,e,f,g,h) in the column, for each storage condition, are significantly different ($p < 0.05$) by one-way ANOVA and Bonferroni post-hoc test. Each trial contained three triplicates of apricots per each treatment

bleached chitosan (1% Bl CS at RT and 0.5% Bl CS at 4 °C) was more effective in maintaining a stable pH of the peaches during both storage conditions. Particularly, at 4 °C, treatment with BSF chitosan, both unbleached and bleached, was better than with commercial chitosan.

Discussion

Film-forming ability of chitosan

After validating their film-forming capability, the chitosan samples from BSF were used for the planned application. Indeed, filmogenic ability is a crucial property that chitosan is required to possess to be applied as coating. The chemical features of the biopolymer itself, however, can have an impact on the properties of this coating. Viscosity and relative molecular weight are among the parameters influencing the characteristics of the coating solution, and they were determined to be the main differences between the chitosan from BSF and the commercial one. Therefore, the expected variations in response of coated fruits might be mainly due to the molecular weight of the chitosan applied. Furthermore, the ability of the chitosan coating to inhibit fruit pathogens in the postharvest storage has been largely investigated. An effective inhibition action of chitosan-based coatings against fungal growth was already assessed in different fruits, such as strawberry [8], tomato [61], papaya [62], pear [63], mango [64], blueberry [65].

Weight loss

Loss of weight occurs in fresh fruits, mainly due to transpiration and respiration processes that cause moisture evaporation between the fruit tissue and surrounding air. Chitosan coating acts as a semipermeable barrier against oxygen, carbon dioxide and moisture, thereby reducing respiration and water loss and counteracting the dehydration and shrinkage of the fruit [66, 67]. In the present work, a significant effect of the BSF-based chitosan coating was observed in all treated fruits, with the exception of peaches, compared to untreated fruits; particularly bleached chitosan was shown to be a better treatment than the unbleached one, already at a 0.5% concentration, mainly at 4 °C. Crustacean-derived chitosan, at a concentration from 0.5% to 2%, have already been effective in reducing weight loss of fresh apricots [68–70], as well as whole and fresh-cut nectarines [71, 72], all stored at cold temperature. The effect of weight loss reduction mediated

Table 4 Results of evaluation of weight loss, TSS content and pH of peaches after 8 days of storage at RT and 16 days at 4 °C

Peaches			
Treatments	**Weight loss (%)**	**TSS (°Brix)**	**pH**
Before treatment		11.3 ± 1.4	4.25 ± 0.08
After storage at RT			
Ctrl -	60 ± 3.1^{a}	27.2 ± 0.3^{a}	4.66 ± 0.40^{a}
Solvent	53 ± 2.9^{ab}	17.8 ± 1.9^{c}	4.79 ± 0.07^{a}
Comm CS 0.5%	55 ± 5.9^{ab}	20.5 ± 2.7^{bc}	4.42 ± 0.00^{a}
Comm CS 1%	55 ± 1.4^{ab}	21.2 ± 1.1^{bc}	4.91 ± 0.00^{a}
Unbl CS 0.5%	47 ± 3.7^{bc}	17.3 ± 1.7^{c}	4.67 ± 0.02^{a}
Unbl CS 1%	41 ± 4.9^{c}	17.8 ± 0.9^{c}	4.57 ± 0.00^{a}
Bl CS 0.5%	40 ± 3.6^{c}	19.1 ± 0.6^{bc}	4.51 ± 0.00^{a}
Bl CS 1%	46 ± 1.2^{bc}	22.9 ± 0.1^{b}	4.39 ± 0.26^{a}
After storage at 4 °C			
Ctrl -	68 ± 2.4^{a}	17.2 ± 2.5^{ab}	4.43 ± 0.02^{c}
Solvent	60 ± 4.2^{b}	20.6 ± 1.2^{a}	5.15 ± 0.03^{a}
Comm CS 0.5%	59 ± 1.9^{b}	20.8 ± 0.7^{a}	4.85 ± 0.01^{ab}
Comm CS 1%	62 ± 1.8^{ab}	19.7 ± 0.6^{ab}	4.65 ± 0.12^{bc}
Unbl CS 0.5%	43 ± 2.1^{c}	16.4 ± 1.5^{b}	4.48 ± 0.04^{c}
Unbl CS 1%	45 ± 2.2^{c}	20.8 ± 0.6^{a}	4.49 ± 0.01^{c}
Bl CS 0.5%	40 ± 1.0^{c}	19 ± 0.5^{ab}	4.47 ± 0.02^{c}
Bl CS 1%	41 ± 1.1^{c}	18.2 ± 1.2^{ab}	4.51 ± 0.12^{c}

Treatments: untreated fruits (Ctrl -), solvent only, coating with pupal exuviae unbleached (Unbl CS), bleached (Bl CS) and commercial (Comm CS) chitosan. Means followed by different letters (a,b,c) in the column, for each storage condition, are significantly different ($p < 0.05$) by one-way ANOVA and Bonferroni post-hoc test. Each trial contained three triplicates of apricots per each treatment

by chitosan coatings was demonstrated for many other fruits, including tomatoes [61], berries [12, 13], citrus fruits [73, 74], grapes [75] and mangoes [76]. On the contrary, no significant effect of the chitosan treatment was detected in cold-stored nectarines by Ramirez et al. [77], in accordance with the present results. Ghasemnezhad et al. [78] found a significative difference in the efficacy of the coating depending on its chitosan concentration: only 0.25% chitosan significantly reduced the apricot weight loss compared to the control and to the higher chitosan concentrations (0.5% and 0.75%). It was assumed that the anaerobic respiration of the fruit could be increased by high concentrations of chitosan, resulting in greater weight loss [78]. This was in agreement with the reported results, in which the BSF-bleached sample performed better at 0.5% chitosan than at 1%. This suggests testing further concentrations of BSF chitosan, below 0.5%, in future experiments.

Variation of TSS content

TSS is an estimation of the sugar content of the fruit, serving as an indicator of fruit ripening. TSS increases during ripening, due to the hydrolytic conversion of starch stored by the fruit into sugars, mainly glucose, fructose, and sucrose [11, 79]. As the fruit reaches full ripeness, the TSS content decreases because of the reduction of ethylene production and respiration rate. As storage further proceeds, with the advancement of the postharvest ripening process, TSS increases again because of the restarting of ethylene synthesis and fruit respiration, with consequent hydrolysis of starch into sugars. This process occurs in "climacteric" fruits (e.g., apricots, peaches, apples, pears), in which the biochemical ripening mechanisms continue even after the detachment of fruit from the plant [80, 81]. In the current experiments, the TSS of apricots, nectarines, and peaches increased during storage, in accordance with other works [69, 71, 72]. On the contrary, other authors reported a decrease in TSS in the same fruits [68, 70, 77, 78, 82]. These differences could be due to the different physiological stages of fruits at the time of the experiment. According to the literature, the increase in TSS could indicate that fruits are either ripening or at an advanced storage phase. The fruits supplied by the grower were at the right stage of ripeness to be marketed [11, 80]. Therefore, it can be assumed that the TSS increased in the first phase of the experiment until full ripeness was reached, followed by a decrease, and then a further increase again in the final storage phase [11, 80]. In addition, TSS rises during storage because of sugar concentration due to moisture loss [83]. In this work, only in fruits stored at RT, BSF chitosan was effective in reducing the TSS increase compared to the negative control. Especially, 0.5% bleached chitosan was the best treatment for all three tested fruits, whereas in nectarines and peaches, even 0.5% unbleached chitosan contributed. Accordingly, in most experiments carried out on apricots and nectarines, chitosan coating did not give different effects to the controls [68, 70, 71, 77, 78]. Only in a few cases, chitosan effectively kept TSS more stable, but combined with modified atmosphere packaging [69, 72]. In some other fruits, chitosan treatment was more effective in reducing TSS variation, including blueberry [12], mango [11], blackberry [13], lemon [74] and pomegranate [84]. The chitosan coating can modify the internal atmosphere of the fruit, with a reduction in the oxygen level and/or an increase in the carbon dioxide level, thus reducing the respiration rate and metabolic activity, and delaying both the accumulation of sugars and the starch degradation [67, 76].

Variation of pH

Organic acids accumulate in the fruit cells during ripening, as they are the main substrate for the enzymes involved in the respiration process of fruits [11, 85]. An

increase in the respiration rate accelerates glycolytic metabolism and tricarboxylic acid cycle, thus increasing the acid content of fruit [81]. Acidity often reduces during postharvest storage because of acid metabolism, which turns acid and starch into sugars [12]. This is in agreement with the increase in pH observed in our experiments. Through this work, it was possible to find that pH was the parameter on which BSF chitosan had the greatest effect. In both peaches at 4 °C and nectarines and apricots at both storage temperatures, all BSF chitosan coatings significantly reduced or avoided the increase in pH compared to both the negative and solvent controls. BSF chitosan also had a significantly better effect than the commercial polymer. Thus, a slowing down of the acidity decrease, due to a deceleration in the acid metabolism of the fruit, is revealed by the buffering in the chitosan-mediated pH variation [12]. Similar results on the same fruits were obtained by Marvdashti et al., Morsy & Rayan and Chiabrando & Giacalone [68, 69, 71]. In other works, in contrast, no differences in pH evolution were found between coated and uncoated apricots or nectarines [70, 71, 79]. An alkalization-reducing effect mediated by commercial chitosan was observed also in different fruits, such as plums, blueberries, and mangoes [11, 12, 86]. The pH can also be affected by the presence of fungal populations, as fungi and moulds can use organic acids as a growth substrate, thus increasing the pH of the fruit [11].

Conclusions

The results of this investigation revealed that BSF chitosan was more effective than the commercially available crustacean chitosan in food preservation, particularly by maintaining more stable some important post-harvest physico-chemical parameters in fresh apricots, nectarines and peaches, especially at room temperature. The coating properties of BSF chitosan could be further improved by acting on the characteristics of the chitosan: chitosan used in the present experiments, with a similar DD, had a lower molecular weight than commercial chitosan, which, according to the literature, might affect the barrier properties of the coating [87, 88].

Significant differences were found between the two tested concentrations of chitosan, and between the bleached and the unbleached polymer. Specifically, bleached chitosan was found to be a better treatment than unbleached one, already at 0.5% concentration; in contrast, unbleached chitosan is more active at the higher concentration. This is probably due to the presence of pigments that hide the activity of the polymer, requiring a higher concentration for the same effectiveness. Based on the experiments carried out, it is clear that chitosan from BSF performed significantly better than solvent treatment alone, underlining the inherently good effect of the biopolymer in slowing down the spoilage of fresh fruit and thus retaining its storage.

Further work could also investigate coatings with different concentrations of chitosan or blending with other natural active components (e.g., starch, proteins, plant extracts) that can enhance its preservative effect. These preliminary results represent an encouraging starting point for the validation of insect biomass for the production of chitosan for use in the agri-food chain. Indeed, with a view to the future, the potential of producing a natural polymer for packaging with this preservation performance turns out to be crucial for both environmental safeguard and human health. Actually, prolonging the shelf life of fresh food reduces food waste, a huge issue of our millennium. Besides that, the bioconverter insect *H. illucens*, being a highly sustainable source and having its breeding always readily available, represents an alternative and valuable source for the production of chitosan, thus obtained in a completely zero-waste circular economy system. The innovation of our work will thus allow, in the future, preserving food, taking care of the environment and also recovering what is normally wasted, thereby becoming a product with high biological value.

Abbreviations

BSF	Black soldier fly
Bl	Bleached
Bl CS	Bleached chitosan
CT	Chitin
CS	Chitosan
Comm CT	Commercial chitin
Comm CS	Commercial chitosan
DD	Deacetylation degree
Ctrl-	Negative control
RT	Room temperature
TSS	Total soluble solids
Unbl	Unbleached
Unbl CS	Unbleached chitosan

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Author contributions

Conceptualization, PF; funding acquisition, PF, data curation, PF, MT, ET, AG, CS; methodology, PF, MT, ET, AG; supervision, PF, CS, RS; writing—original draft, PF, MT, ET, AG, CS; writing—review and editing, PF, MT, ET, AG, DI, CS, RS, TH, SZ. All authors have read and agreed to the published version of the manuscript.

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

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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