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Chitin and chitosan materials from black soldier fly (*Hermetia illucens*): An insight onto their thermal degradation and mechanical behavior linked to their copolymeric structure

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ABSTRACT

Chitin and chitosan from black soldier fly (*Hermetia illucens*) rearing, associated with the different development stages (larvae, pupal exuviae and adults), were characterized by ATR-IR determining the R_{AC} ratio, associated with the acetylation degree of the polymers. It was possible to compare the different samples, and it was detected that the chitin from adult insects was the one with the highest acetylation degree. Discoloured chitin samples were also tested, and they showed a lower acetylation degree than the not discoloured ones. Chitosan samples, obtained by heterogeneous deacetylation, from larvae and pupal exuviae were the most deacetylated and they resulted similar to commercial chitosan from shrimps. The characterization of samples by thermogravimetry in nitrogen was carried out and their thermal behaviour resulted different for chitin and chitosan samples. For chitosan samples an additional mass loss at lower temperature could be revealed with respect to chitin, and its quantitative value decreased by increasing the R_{AC} ratio. It could be deduced that the chitosan samples from *Hermetia illucens* (HI) were mainly blocky, except for the chitosan sample obtained from larvae and the commercial chitosan from shrimp. For the latter samples a mainly homogeneous distribution of acetamide groups on the polymer backbone could be hypothesized. The mechanical properties of films obtained by solvent casting of acidic water solutions of chitosan samples were measured. The highest values for tensile strength and elongation at break were observed for the film obtained by chitin from *H. illucens* larvae or from commercial chitosan from shrimps. Interestingly, these chitosan samples are those corresponding to a homogenous distribution of acetyl groups, suggesting that this feature can play a significant role in influencing the mechanical properties of chitosan films.

1. Introduction

Highly abundant chitinaceous waste can be considered a renewable source for new advanced biobased and circular materials [1,2]. Functional materials obtained from chitin, like chitin nanofibrils and chitosan, were demonstrated to show anti-microbial and anti-inflammatory activities [3,4], thus their use in packaging [4,5], hygiene [6,7], personal care [8], cosmetic [9,10] and biomedical [11,12] applications is rapidly increasing [13]. Recently, anti-oxidant properties were also observed for chitosan, suggesting its potential use in applications where resistance to oxidative stress is required [14–16].

In this context, in view of further growth, obtaining chitin and chitosan from different sources can be a very good strategy [17–19], hence researchers developed applications considering chitin and chitosan from different terrestrial (fungi and insects) [20,21] and marine (mainly crustaceans) sources [18]. In recent years, valuable protein sources, necessary for aquaculture, were identified in biotechnological processes like the rearing of insects [22]. Their development is particularly useful, as they can behave as bioconverters [23], transforming agro-food waste into proteins, chitin (present in their exoskeleton) and other valuable compounds [24]. Black soldier fly (*Hermetia illucens*) rearing, in particular, was demonstrated as a highly effective methodology to develop

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novel agro-industrial chains inspired by a circular economy approach [25]. Farms of bioconverter insects, indeed, are currently spread worldwide for industrial protein feed production and organic waste management, and pupal exuviae and dead adults are the only waste of this process, and they are very rich sources of chitin. Chitin can be considered a high value derivative of this process and its exploitation for new productive cycles are effectively a further step of the bioconversion in the circular economy process, from a zero-waste point of view. Insects are an innovative, renewable and sustainable source of chitin and the recovery and the usage of chitin from the side stream of their rearing represent the missing link of a great example of zero-waste circular economy. Currently, numerous studies are exploring the potential of insects as a promising source of novel bioactive compounds and biomass with high biological and economic value. Insects represent one of the richest yet least explored reservoirs of valuable natural substances, including biopolymers, such as chitin and chitosan. Chitin constitutes approximately 25–60 % of the insect cuticle on a dry weight.Chitin is obtained by *Hermetia illucens* (HI) through a demineralization step followed by a deproteinization process. Chitosan is obtained by deacetylation of chitin [16] and it is purified by reprecipitation from acidic solution to eliminate the presence of residual chitin. Both chitin and chitosan can be defined as copolymers of N-acetyl-D-glucosamine and D-glucosamine, linked by covalent β-(1 → 4)-linkages (like the linkages between glucose units forming cellulose) [26] but, in the case of chitin, the major comonomer is N-acetyl glucosamine, whereas, in the case of chitosan, the major comonomer is glucosamine [27] (Fig. 1).

Insect-derived chitosan exhibits properties comparable to, and in some cases exceeding, those of traditional sources, particularly in terms of biocompatibility, antimicrobial activity, and biodegradability—key attributes for biomedical applications [28,29].

Some researchers investigated the structure of chitosan obtained by different methodologies by spectroscopic methods and adopting parameters linked to the random, alternating or block distribution of repeating units in the polymer backbone [30]. In particular, it was reported that the production of chitosan can be carried out through heterogeneous or homogeneous methods [16,31]. The latter is more time consuming and generally results in chitosan with a lower deacetylation degree. Moreover, the glucosamine repeating unit typical of chitosan as well as the residual N-acetyl-glucosamine units are distributed in a completely random way and thus homogeneously along the chain. The heterogeneous method, which is faster, results, on the contrary, in a deacetylation driven by hindering elements (accessibility of polymer chains), with chitosan preserving chitin blocks and a tendency to develop a nanofibrillar morphology. In fact, chitin nanofibrils are produced by a mild deacetylation, that produces chitosan glucosamine units only on nanofibrils surface [32]. The heterogenous acetylation, modifying less the chitinous crystal fraction of the material, results in a chitosan more similar to the original nanofibrillar chitin. These aspects linked to the possibility of controlling repeating units' distribution are significant [33]. After all, chitin-chitosan block oligomers were

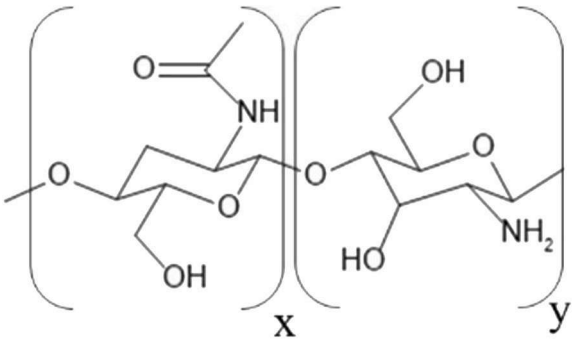

Fig. 1. N-acetyl-D-glucosamine and D-glucosamine copolymeric units of chitin and chitosan.

considered very important, and studies regarding their synthesis were recently attempted [34–38]. This tendency to obtain a blocky structure by heterogeneous deacetylation is attributed to the lower efficiency of the hydroxide anions during deacetylation in hydrolysing acetyl groups, due to the presence of not completely dissolved chitin crystals. This study opened the possibility of further exploiting and controlling chitosan from HI structure and properties. Certainly, it confirmed that the heterogeneous method is currently the best option in view of an industrial up-scale of chitosan production from insects. Nevertheless, it should be mentioned that very recently, the use of natural deep eutectic solvents for the chitin extraction was also investigated [39,40] as an alternative to the traditional method based on demineralization, deproteinization and bleaching (discoloration). The authors suggested that this methodology for chitin extraction, applied to HI, can facilitate successive chitosan production. Although these findings could be promising, the cost of solvents and the possible difficulties in their complete elimination after extraction suggest the necessity of further research in this field. Then, the process based on acidic and basic treatments to obtain chitosan from HI following the demineralization, deproteinization and heterogeneous deacetylation (possibly preceded by discoloration) steps are still the most reliable [41].

Chitosan production from insects has been reported almost exclusively through chemically heterogeneous deacetylation, typically using sodium hydroxide as the deacetylating agent. Chitosan yields based on the original insect biomass range from 2 % to 8 %, with exceptionally high yields (26–28 %) reported by Song et al. [42] from *Chrysomya megacephala* larvae and by Luo et al. [43] from cicada sloughs. When calculated from the dry weight of extracted chitin, chitosan yields range between 60 % and 83 %. For comparison, yields obtained from crustaceans using heterogeneous deacetylation range from 4 % to 15 % based on the initial dry biomass [43–45] which are slightly higher than those from insects. This difference is primarily attributed to the higher protein and fat content in insect biomass [46]. Chitosan yields are influenced not only by the purification method but also by species and harvest time [47].

The degree of deacetylation (DD) of insect-derived chitosan obtained through heterogeneous treatment varies between 62 % and 98 %, with the only notably lower value (57 %) reported by Monter-Miranda et al. [48] for chitosan extracted from *Brachystola magna* adults. In crustaceans, with heterogeneous deacetylation it typically possible to obtain a DD ranging from 56 % to 98 % [49] while the homogeneous methods generally produce chitosan with a lower DD of 48–55 % [47].

The molecular weight of chitosan from crustaceans usually ranges from 100 to 1000 kDa [44,50], whereas insect-derived chitosan shows greater variability, ranging from 26 to 300 kDa. Very low molecular weights (3 and 7 kDa) have also been reported [51–53]. Species-specific factors also appear to influence molecular weight, as demonstrated by Kim et al. [54] applying the same deacetylation conditions as Kaya et al. [51], but obtaining a significantly higher molecular weight (308 kDa from adult crickets compared to approximately 3 kDa from adult and larval *Leptinotarsa decemlineata*). Exceptionally high molecular weights (3290–5900 kDa) have been reported by Paulino et al. [55] for chitosan produced from silkworms using 40 % sodium hydroxide and sodium borohydride at 100 °C. This outcome may be attributed to the ability of sodium borohydride to prevent oxidative cleavage of glycosidic bonds during the deacetylation process.

A fast methodology for characterizing chitin, chitosan and chitin nanofibrils is Attenuated Total Reflectance (ATR) infrared spectroscopy, a non-destructive technique based on recording the spectrum of a sample in contact with a crystal having a high refractive index [56]. This technique allows spectra to be obtained also from solid samples, without any preliminary preparation, saving time and allowing the analysis of non-soluble systems such as chitin. Spectra were strongly dependent on the adhesion between sample and crystal but, if the samples to be characterized are powdery, generally it is possible to obtain good quality spectra and a sufficient representativity for composition of the

powder-based sample. Additionally, characterization methodologies based on ATR-IR can be compatible with in-line monitoring processes and with artificial intelligence applications [57]. Infrared analysis in transmission was in general adopted by several researchers to compare chitins, chitin nanofibrils and chitosan from different sources [57,58]. Many papers, where the conversion of chitin to chitosan was considered, reported a reduction of amide I band of acetamide groups, that showed a well-defined peak at 1650 cm^{-1} with a minor shoulder at 1625 cm^{-1}, in more deacetylated products, thus revealing a conversion of chitin to chitosan [57]. Then this technique was used by several researchers to determine the deacetylation degree of chitin based on transmission spectra [59–64]. Triunfo et al. [65] evidenced that the acetylation degree determined by potentiometric analysis was in agreement with the trend of acetylation degree determined by elaborating transmission spectra.

Thermal properties of chitin and chitosan were also considered to characterize them. The mechanism of thermal degradation consists of the production of ammonia from the D-glucosamine unit and this degradation path is typical of chitosan [66]. The N-Acetyl glucosamine unit typical of chitin results in the evolution of acetic acid at higher temperature [67]. Thanks to these distinguished steps, it is reported that through DSC measurements it is possible to determine the acetylation degree of chitin and chitosan by considering the area or the height of the endothermic peak associated with the loss of acetic acid [68]. Thermogravimetric investigation related to chitin and chitosan were carried out by several authors [69], but mainly onto chitin and chitosan from crustaceans and these studies were never addressed at correlating thermal behaviour with the development stages of black soldier fly.

Mechanical properties of chitin or chitosan can be determined by testing the properties of films. Chitosan films are widely investigated because they could be used in packaging or coating applications. Hence the preparation and characterization of films is significant from an industrial point of view. The preparation of films from chitosan is possible by dissolving it in 1 % acetic acid [70]. In fact, chitosan -NH_2 groups are protonated, and the polymer becomes water soluble as positive ionomer. In this case the presence of acetate anions will grant the material electroneutrality. Only De Masi et al. [71] have characterized chitosan films in terms of their tensile properties, whereas Panariello et al. [72] considered blends of chitosan and chitin nanofibrils. In both papers, commercial chitosan from shrimp was considered. A similar characterization should be carried out onto chitosan from HI, to give an insight into its possible application as film in several sectors, for instance packaging.

In the present paper an ATR-IR methodology was adopted to compare the degree of acetylation of chitin and chitosan obtained in the different biomasses (larvae, pupal exuviae, adults) of the black soldier fly insect to distinguish N-acetyl glucosamine (chitin based) from acetyl glucosamine (chitosan based) copolymers obtained by an heterogeneous deacetylation method. Moreover, the different samples were investigated by thermo-gravimetry to correlate the structural features to the thermal degradation behaviour of samples. Commercial samples of chitin and chitosan from shrimp and mushrooms were also considered for comparison. Mechanical properties of films obtained by solution casting in acetic acid were also determined correlating with their chemical structure and comparing the different samples in terms of their mechanical performances with the aim of evaluating their potentialities as films or coating layers in packaging, agricultural or personal care applications.

2. Material and methods

2.1. Preparation of samples

The extraction of chitin from insect samples (larvae, pupal exuviae and dead adults) was carried out based on the process reported by Hahn et al. [73], as modified in Triunfo et al. [65] called more recently heterogenous method. The method consists of three steps. Briefly, the first is demineralization, to remove minerals, mainly calcium carbonate. The second step is deproteinization. In this step proteins were removed from demineralized samples by treatment with 2 M sodium hydroxide (NaOH) (solid:liquid ratio 1:10 (m/v)), stirring for 2 h at 80 °C. The third step is bleaching (often indicated as discoloration).

Chitosan was produced through the heterogeneous deacetylation of both unbleached and bleached chitin derived from three *H. illucens* biomasses (larvae, pupal exuviae, and dead adults). The chitin samples were mixed with 12 M NaOH (Sigma-Aldrich, St. Louis, Missouri, USA) at a solid-to-liquid ratio of 1:20 and stirred for 4 h at 100 °C. After the reaction, the suspension was filtered using filter paper, and the solid residue was washed with distilled water until neutral. The deacetylated material was then incubated in 1 % (v/v) acetic acid (Sigma-Aldrich, St. Louis, Missouri, USA) at room temperature for 48 h with stirring. The mixture was centrifuged at 10,000 rpm for 5 min, and the supernatant was collected.

The solution was adjusted to pH 8 with 6 M NaOH (Sigma-Aldrich, St. Louis, Missouri, USA) and incubated overnight at 4 °C to precipitate the solubilized chitosan. The suspension was centrifuged again, and the chitosan was collected and washed with distilled water to remove any remaining acetate. The final product was freeze-dried (−20 °C) and stored at room temperature (25 °C).

Chitin samples are indicated in this paper by using as Chiti-A, Chiti-EP or Chiti-L for indicating materials obtained from adults, pupal exuviae and larvae, respectively. Chiti-FUN and Chiti-SH are commercial Glentham products obtained from Fungi (GC8934) and Shrimps (GC0425), respectively. Chitosan samples are indicated by Chito prefix using the same suffixes and a chitosan from shrimp (Chito-SH) (Glentham, GP8523) was also characterized for comparison. The D letter following the suffix indicates that a discoloration step was also carried out.

Chitosan films were prepared by dissolving chitosan samples in acetic acid 1 % to obtain a solution at a concentration of 10 mg/mL and pouring them into rectangular plates. Then the films were dried at 25 °C.

2.2. Infrared -ATR characterization

The powder of chitin or chitosan was homogenized and reduced in dimension in a mortar using a pestle. Then the powder is transferred from the mortar to the ATR crystal. Infrared spectra were recorded in the 550–4000 cm^{-1} range with a Nicolet 380 Thermo Corporation Fourier Transform Infrared (FTIR) Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with smart Itx ATR (Attenuated Total Reflection) accessory with a diamond plate, collecting 256 scans at 2 cm^{-1} resolutions. EZ ONMIC software (OMNIC 7.2, Thermo, Waltham, MA, USA) was used to elaborate the spectra and to compare different spectra profiles. Three spectra were recorded for each sample, each acquired from a different aliquot of chitin or chitosan powder.

The R_{AC} ratio, that can be correlated to the acetylation degree of the sample [74], was determined by Eq (1)

$$R_{AC} = \frac{A_{1620}}{A_{1020}} \quad (1)$$

where A_{1620} is the area of the band obtained by integrating the peak at 1620 cm^{-1} in the range 1695-1618 cm^{-1} and A_{1020} is the area of the reference band in the range 1184-1024 cm^{-1}. The integrations were carried out after tracing a baseline passing through the minima present in all the spectra at about 1735 cm^{-1} and 1185 cm^{-1}, by using a EZ OMNIC software. The values of R_{AC} were averaged considering the three recorded spectra and the standard deviation was then calculated for each sample.

Chitosan films were characterized by ATR-IR adopting the same equipment settings. All the samples were characterized by collecting spectra on both film sides, to control possible compositional

inhomogeneities. In all the cases the spectra obtained on the different sides resulted identical.

2.3. Thermogravimetric characterization

Thermogravimetric analysis was performed with a PerkinElmer Pyris TGA 4000 thermogravimetric analyzer at a heating rate of 10 °C/min under nitrogen purge (30 mL/min) from room temperature to 600C, then with air as purge gas up to 900 °C. About 10 mg of each sample was used for the analysis. The software Pyris was used for thermograms elaborations.

2.4. Mechanical tests

The quasi-static tensile tests (ASTM D882) on the films were performed at a deformation rate of 10 mm/min using an Instron model 5500R universal testing machine (Canton, MA, USA) equipped with a 10 N load cell. The machine was interfaced with MERLIN software (INSTRON version 4.42 S/N-014733H), and pneumatic grips were used. Tensile tests were conducted on 80 × 15 × 0.010 mm strips with a gauge length of 50 mm. The strips were obtained by accurately cutting chitosan films. At least 10 specimens were tested for each sample, and at least 6 values were averaged except for pupal exuviae films. For these films only four values were averaged. In fact, because of their fragility, the recording of the curves was difficult.

The elastic tensile modulus was measured using dynamic mechanical thermal analysis (DMTA) performed on a Gabo Eplexor (Gabo, Ahlden, Germany) with a 100 N load cell. At least three before mentioned film specimens were tested. During the test, the temperature and frequency were maintained at a constant 25 °C and 1 Hz, respectively.

3. Results

Infrared ATR spectra were recorded for all the chitin and chitosan powder samples (Fig. 2). The characteristic peaks of chitin (Fig. 2a and c) can be observed at 1310–1320 cm^{-1} (C-N stretching, amide III), 1550–1560 cm^{-1}, (N-H bending, amide II), 1650–1655 cm^{-1} (C=O stretching, amide I), 3100–3110 cm^{-1} (N-H symmetric stretching), 3255–3270 cm^{-1} (N-H asymmetric stretching) and 3430–3450 cm^{-1} (O-H stretching) [48]. Chitin samples obtained from the different biomasses of HI showed very similar spectra. The spectra closely resembled those of commercial chitin derived from shrimp and fungal sources (Fig. 2a), in good agreement with the observations of Triunfo et al. [65], that have carried out an infrared characterization in transmission conditions. However, the bands at 3100–3110, 3255–3270 and 3430-3450 cm^{-1} seem more overlapped in the HI samples, indicating a slightly more complex chemical structure, attributable to a different structure of the chitin fibrils but also to the different extraction and purification methods. Spectra of the discolored chitin samples (Fig. 2c) resulted like the ones of not discolored ones, but the spectra resulted much more similar to the ones of commercial chitin derived from shrimps and fungi.

Chitosan, being derived from the incomplete deacetylation of chitin, displays spectral features that closely resemble those of chitin (Fig. 2b and d) [75]. Nevertheless, peaks related to acetyl groups are obviously less intense because of the conversion of acetamide groups into amine groups. In terms of peaks wavenumbers and shape, no significant differences can be observed between not discolored and discolored samples.

The values of the R_{AC} were calculated for all the samples and this rapid methodology was found successful to distinguish chitin from chitosan. In fact, all the chitin samples have a R_{AC} above 0,3, whereas all the chitosan have a R_{AC} below 0,25 (Fig. 3). Chitin extracted from adult

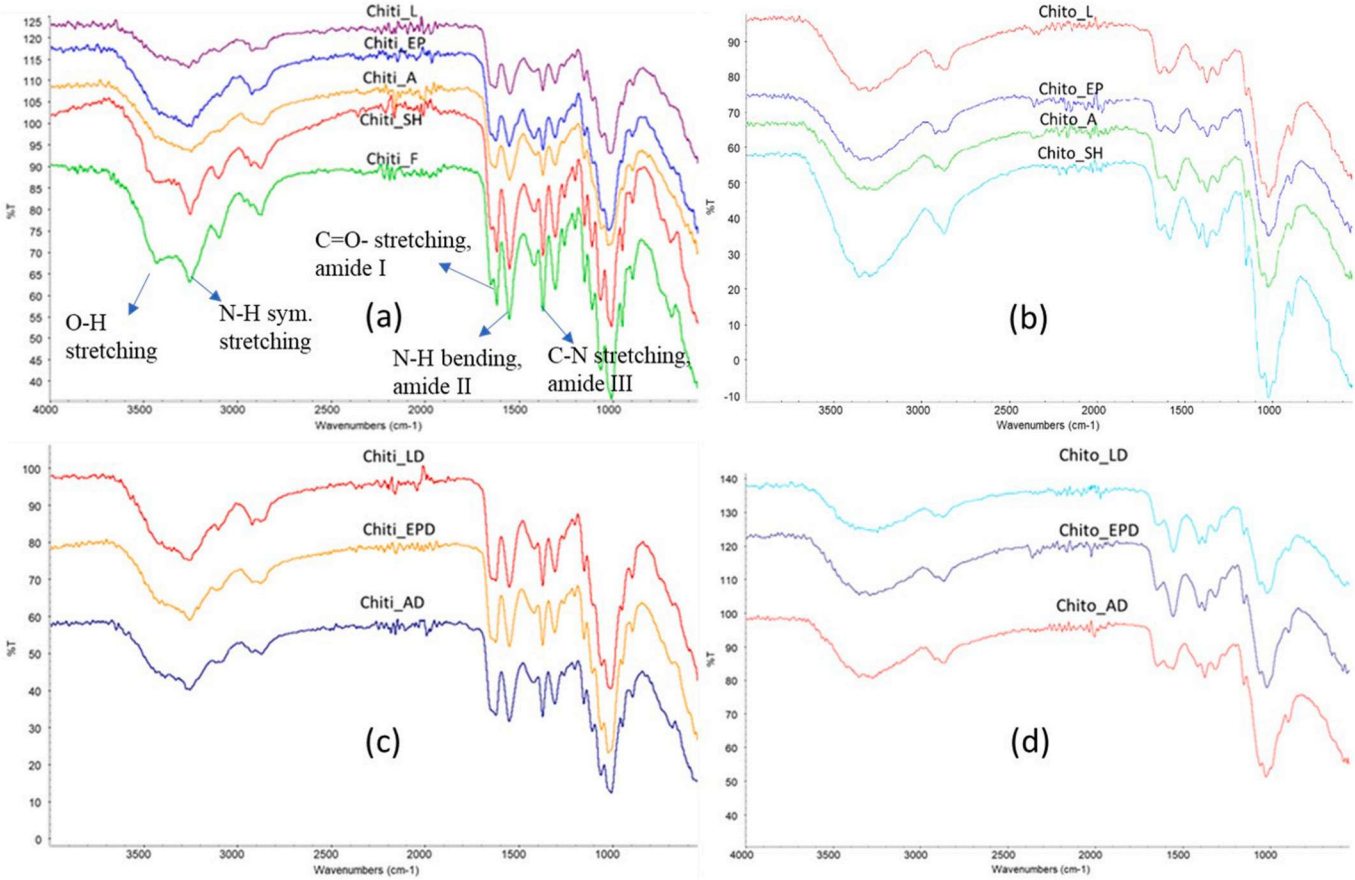

Fig. 2. ATR-IR spectra of not discolored and discolored chitin (a, c) and chitosan (b, d).

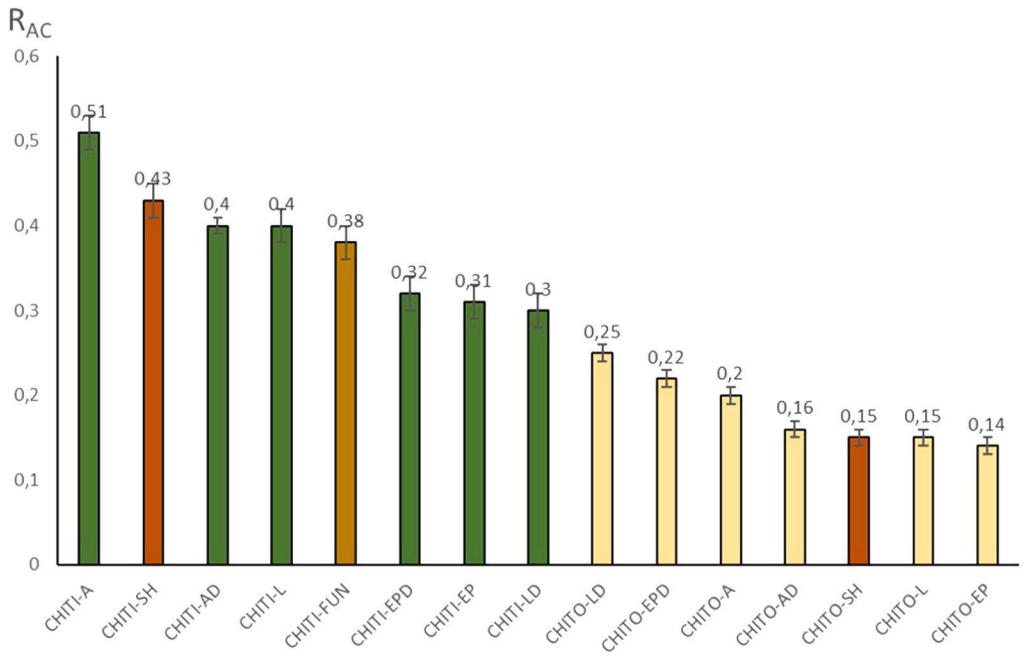

Fig. 3. Comparison between R_{AC} values of chitin and chitosan samples. HI chitin samples were represented by the green bar, HI chitosan samples were represented by the yellow bars and reference chitin and chitosan bars were red for shrimp-based samples of chitin and chitosan and brown for fungi-based chitin.

insects is the most highly acetylated, with measured values indicating a higher degree of acetylation than that observed in commercial samples derived from shrimp and fungiIn good agreement with previous investigations [49], it was found that the acetylation degree of HI chitin is higher for the adults with respect to the larval stage, and it is the lowest considering pupal exuviae. The discoloured samples showed a lower acetylation degree than the not discoloured ones. This can be attributed to the acidic nature of the bleaching treatment, that can induce some deacetylation, especially on the more exposed surfaces of nanofibrillar chitin.

Regarding chitosan samples, it can be noticed that chitosan samples from larvae and pupal exuviae are the most deacetylated. In general, for the discoloured samples the deacetylation is apparently less effective. To explain this result, it should be considered that the deacetylation may be more effective on chitosan having a higher acetylation degree, as yet observed elsewhere [24]. In fact, in the case of a more deacetylated chitin, hydrogen bond formation and nucleophilic interactions are enhanced, above all in the more disordered amorphous fraction of chitin because amine groups have a higher nucleophilic character of the nitrogen with respect to nitrogen in N-acetyl groups. Because of this competition, hydroxide anions coming from NaOH during the deacetylation is less effective with respect to a chitin with a higher acetylation degree. In good agreement with this explanation, the highest decrease in R_{AC} passing from chitin to chitosan, was observed for the chitin from adult HI, whereas the lowest differences were observed for EP, EPD and LD.

In order to use chitosan and chitin from HI in industrial applications the resistance to heating should be investigated. With this purpose, the different samples were characterized by thermogravimetry in nitrogen, to study their stability to heating. Interestingly, it was found that in chitin samples two main mass losses can be seen in the thermogram, as revealed by the derivative DTG curves (Fig. 4b) [67,76,77]. The first one, in the temperature range 40–150 °C (Table 1), is attributable to loss of humidity, the second one, occurring above 330 °C and showing an inflection point in the range 368–402 °C in the different samples, is attributable to the decomposition of the poly(N-acetyl- glucosamine). Barbosa et al. [67], that analyzed by infrared spectroscopy the gases evolved during the thermal degradation experiments, have shown that significant acetamide and acetic acid loss can be observed for decomposition of chitin. However, they did not notice ammonia evolution in chitin samples. In our samples the final residue was found in the range 16–21 % by weight.

Regarding the different development stages of HI, the highest thermal stability was shown by chitin from pupal exuviae, showing an onset temperature of 354 °C and an inflection point of 401 °C. The stability of chitin followed the trend: CHITI-EP > CHITI-A > CHITI-L. Since the acetylation degree follows the order CHITI-A > CHITI-L > CHITI-EP as well as the crystallinity [65], a correlation between this parameter and chitin thermal stability cannot be invoked. Probably, the thermal behavior can be influenced by the presence of impurities, that may catalyze the beginning of degradation, and are reported to be more abundant in the adult chitin samples [65]. In good agreement the discolored chitin from adults showed an increased inflection point with respect to the not discolored sample. Chitin from HI revealed a thermal behaviour similar to commercial chitin from shrimps and fungi. In general chitin showed a very good thermal stability, and insect chitin from pupal exuviae resulted more stable than fungi and shrimp sourced chitin.

Regarding chitosan samples, as yet reported by several authors [77], they showed three main mass loss steps. In fact, an additional mass loss step is found in the range of inflection point 289–312 °C and can be associated with the glucosamine unit of the polymer, hence to the chitosan fraction of the samples. Except for CHITO-SH, CHITO-L and CHITO-FUN, chitosan samples showed both a mass loss associated with chitosan at lower temperature and a mass loss associated with chitin at a higher temperature. The mass loss associated with the two different steps was calculated and reported as a function of R_{AC} (Fig. 5). The values of weight loss associated with chitin were completely different compared to chitosan and chitin samples (Fig. 5a). For chitin samples the values were similar and in the range 65–80 % by weight. In the case of chitosan, the weight loss associated with chitin was significantly lower. Chitosan from adults, discolored or not, showed the highest values of chitin weight loss, where CHITO-L showed a zero-value associated with chitin mass loss. Pupal exuviae showed an intermediate value.

The weight loss associated with commercial CHITO-SH is very

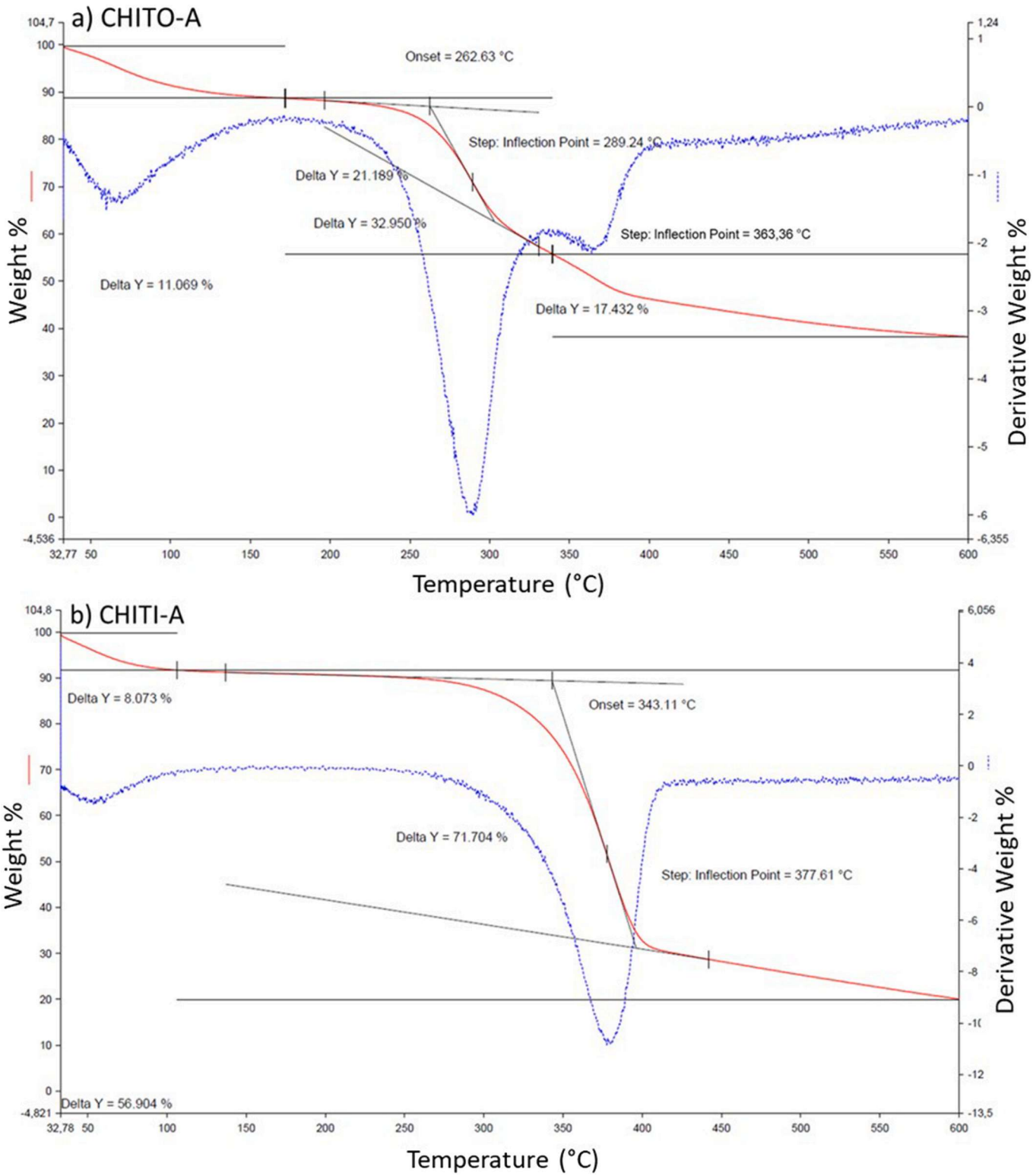

Fig. 4. Representative thermograms (red line) and DTG curves (blue line) of (a) CHITO- A and (b) CHITI-A. Onset, inflection point and weight loss steps were evidenced in the figures.

similar to the one observed for CHITO-EP, moreover it was found that the weight loss decreased by increasing the R_{AC} value (Fig. 5b), indicating the dependence from the concentration of glucosamine units in the polymer. Nevertheless, the trend is not linear and at higher values of R_{AC} the decrease becomes less evident. Pure chitosan was found to produce at high temperature evolution of acetamide and ammonia [67]. Then, these results can be tentatively explained considering the decomposition of chitosan following the production of inorganic (ammonia) or organic (amide) nitrogen compounds. The mechanism generating ammonia is probably favored when the acetylation degree is very low (resulting in higher values of chitosan mass loss at low R_{AC} values), whereas the mechanism generating amide compounds is

Table 1
Results of thermogravimetric tests performed in nitrogen flow on chitin and chitosan samples.

Weight loss	Onset (°C)	Inflection point (°C)	Inflection 1point 2(°C)	Weight loss	Weight loss chitin(%)	Residue(%)	Rac
water (%)				chitosan (%)			
CHITI-A 8.073	343.11	–	377.61		71.704	20.223	0.507 ± 0.017
CHITI-AD 4.878	340.94		382.24		79.230	15.892	0.401 ± 0.007
CHITI-EP 6.608	353.94	–	401.09		72.699	20.693	0.309 ± 0.015
CHITI-L 4.917	334.87	–	368.73		77.417	17.666	0.403 ± 0.015
CHITI- 9.243	342.16	–	385.58		71.944	18.813	0.384 ± 0.019
CHITI-SH 10.043	341.06	–	382.83		72.519	17.438	0.434 ± 0.020
CHITO-A 11.069	262.63	289.24	363.36	32.950	17.432	38.549	0.197 ± 0.009
CHITO- 10.083	278.81	300.98	384.59	37.487	17.969	34.461	0.158 ± 0.008
CHITO-EP 10.017	261.25	291.65	380.92	47.881	10.105	31.997	0.135 ± 0.008
CHITO- 15.490 SH	277.65	303.45	–	53.671	–	30.839	0.147 ± 0.010
CHITO L 12.303	289.877	312.130	–	43.891	–	34.858	0.151 ± 0.006
CHITO LD9.933	275.537	302.167	386.833	30.949	15.176	35.168	0.251 ± 0.008
CHITO 14.418 EPD	279.062	304.500	390.833	30.142	10.443	35.810	0.217 ± 0.008

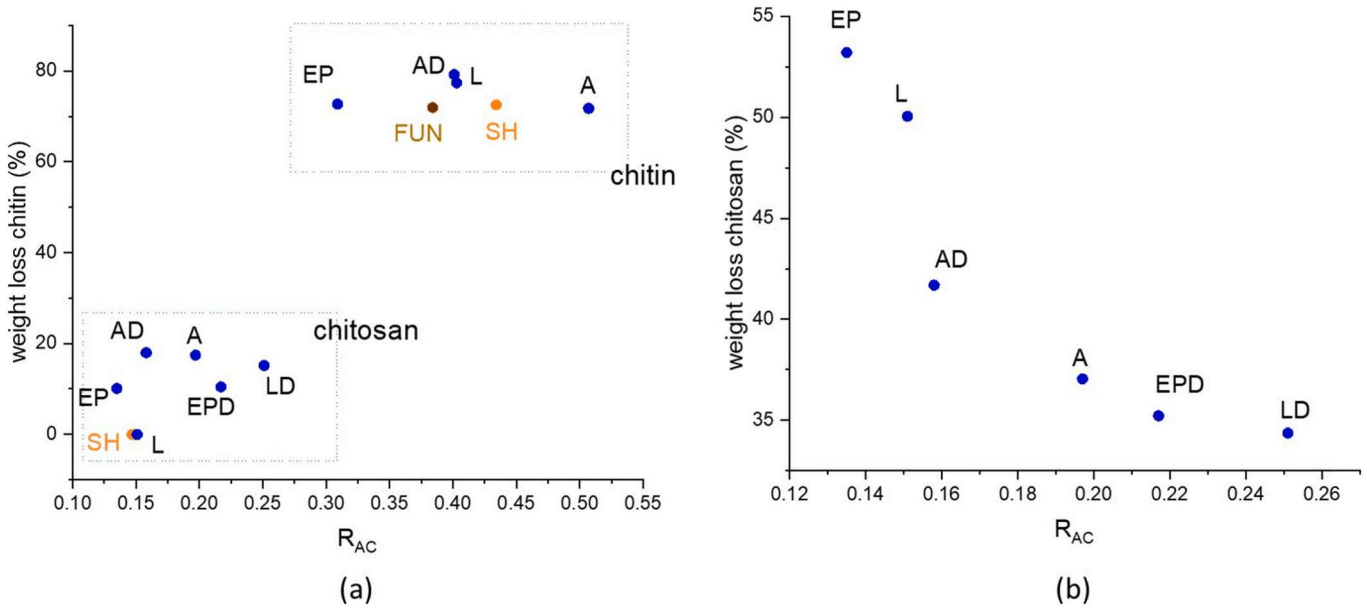

Fig. 5. Trend of the weight loss attributed to (a) chitin and (b) chitosan as a function of R_{AC}.

anyway present.

Moreover, it was evidenced that a pure chitosan sample, having an acetylation degree very close to zero, did not show any evolution of acetic acid [67]. The evolution of acetic acid is thus associated with the N-acetyl glucosamine units, whereas the evolution of ammonia can be associated with glucosamine units. Interestingly, the evolution of acetamide was revealed in the degradation of both pure chitosan and chitin, reasonably because its evolution is linked not only to the decomposition of acetyl amide groups, but also to the fragmentation of the polymeric chain. In good agreement with our results, Corazzari et al. [78], that analyzed both chitin and chitosan samples having different values of acetylation degree, suggested a correlation between the total weight loss due to acetic acid formation and the acetylation degree. Interestingly, the thermal decomposition of chitin and chitosan nanofibers was examined by TGA and DSC [79]. In good agreement with our results, the maximum decomposition temperature of the chitin nanofibers was higher than that of the chitosan nanofibers, and this difference is attributed to the higher thermal stability of the N-acetyl units. The separate decomposition of glucosamine and N-acetyl glucosamine units was investigated also by DSC and two separate exothermic peaks were detected. The authors associated these results to the block structure in the chitosan backbone due to preparation of chitosan occurred by heterogeneous deacetylation. As an heterogeneous deacetylation approach was followed also in our work, and the presence of these two separated peaks in TGA thermograms can be considered a confirmation of having obtained a blocky structure for our poly(N-acetyl-glucosamine) (glucosamine) copolymers, as yet discussed in our previous work [65].

The CHITO-L sample, obtained from larvae, is the one not showing the presence of a mass loss associated with chitin, thus it is reasonable to hypothesize the presence of N-acetyl glucosamine blocks is limited and the distribution of acetyl groups on the chain is almost homogeneous. Interestingly, the block structure, preserving N-acetyl glucosamine blocks, depends on the occurring of deacetylation and it was found that a chitin pre-treated by strong basis for a long time is generally deacetylated resulting in a less blocky copolymer because of the better accessibility of OH^- to acetamide groups [65]. In our chitin samples the morphology complexity, investigated by SEM, was found in the order: CHITI-A > CHITI-EP > CHITI-L [65]. Then the most disordered and thus most accessible chitin from larvae can be associated with the achievement of a less blocky copolymer, in agreement with our TGA results. In general, the TGA characterization showed the possibility of being exploited for rapidly classifying chitosan samples based on their blocky

or random features.

Chitosan samples produced from HI were used for producing films by solvent casting. The solvent selected was water acidified with acetic acid, since these casting conditions were reported to be advantageous in terms of mechanical properties of films by several researchers [70,72,80]. The chemical composition of our films is thus no more ascribable to chitosan but to its polycation bearing -NH^{3+} groups having a positive charge, that are counterbalanced by acetate anions. Considering that pkA of chitosan is reported to be between 6 and 6.3 [26] and that a concentration of 1 % wt. of acetic acid in water was used (pH about 3), the complete protonation of the -NH_2 groups of chitosan can be reasonably hypothesized. This condition leads to the maximum solubility in water, that can allow highly homogeneous films to be obtained.

The analysis by ATR-IR of the chitosan films showed evident variations with respect to the chitosan powder due to the different composition (Fig. 6). In fact, the spectrum recorded on the film showed additional bands at 2934 cm^{-1}, attributable to C-H stretching of acetate anion. Moreover, the acetate anion C=O asymmetric and symmetric stretching bands at 1540 and 1407 cm^{-1} can be revealed, respectively [81]. A similar comparison was made for all the chitosan films and analogous conclusions were achieved comparing the different chitosan samples spectra with the respective spectra of films (Fig. 7).

Regarding the colour of the films, the HI chitosan films resulted all slightly coloured. The film obtained by using adult HI chitosan was the darkest one, showing a brown colour, whereas the chitosan from discoloured larvae chitin resulted in the least dark and the most transparent (Fig. 8a). However, a more slightly yellowing colour was observed with respect to commercial chitosan from shrimp SH.

The results of the experimental tests showed jagged stress-strain curves, indicating multiple fracture points as evidenced in the comprehensive Fig. 8b. When comparing the films to commercial chitosan SH, the most similar properties were observed in the chitosan film from larvae L, with comparable values of tensile strength, elongation at break, and a slightly higher elastic modulus.

Analysing all the mechanical properties and comparing them in the histograms of Fig. 9, regarding the films obtained from pupal exuviae, they resulted very fragile. Except for the adult specimens, both larvae and exuviae showed improved mechanical properties in the non-discoloured films. In fact, generally, mechanical properties depend significantly on the molecular weight of the polymers and the discoloration was demonstrated to result in a significant decrease in molecular weight [65]. Generally, tensile strengths of chitosan films can vary depending on production conditions, material characteristics, and testing methods employed. However, chitosan films typically exhibit tensile strengths in the range of 20–50 MPa, so the films tested in our work are completely in line with reference chitosan film properties [82]. It is important to note that these values may vary significantly depending on the specific chitosan film formulation, preparation methods, and testing conditions. It can be observed from Fig. 9 that the experimental and pioneering nature of the processing and production methods used for these films did not allow for the duplication of numerous replicates for testing the EP and EPD materials. As a result, the deviations from the mean of the properties derived from the tensile data are quite large. Nevertheless, the average values provide a realistic indication of the mechanical behavior of these films and allow for a meaningful comparison with commercial chitosan.

The reason for the fragility shown by the chitosan films is linked to the presence of ionic interactions and hydrogen bonds. These kinds of bonds result in a material with a low capacity to deform, thus with a limited ductility, with a glass transition higher than room temperature. The plasticization, resulting in the introduction of water [83] or less polar compounds [84] in between chitosan macromolecules, was indicated as a general methodology to improve film flexibility mainly acting on ions mobility. However, the production of films by solvent casting can be difficult in an industrial environment, where plastic films are generally produced by melt processing (for instance by flat die or blow

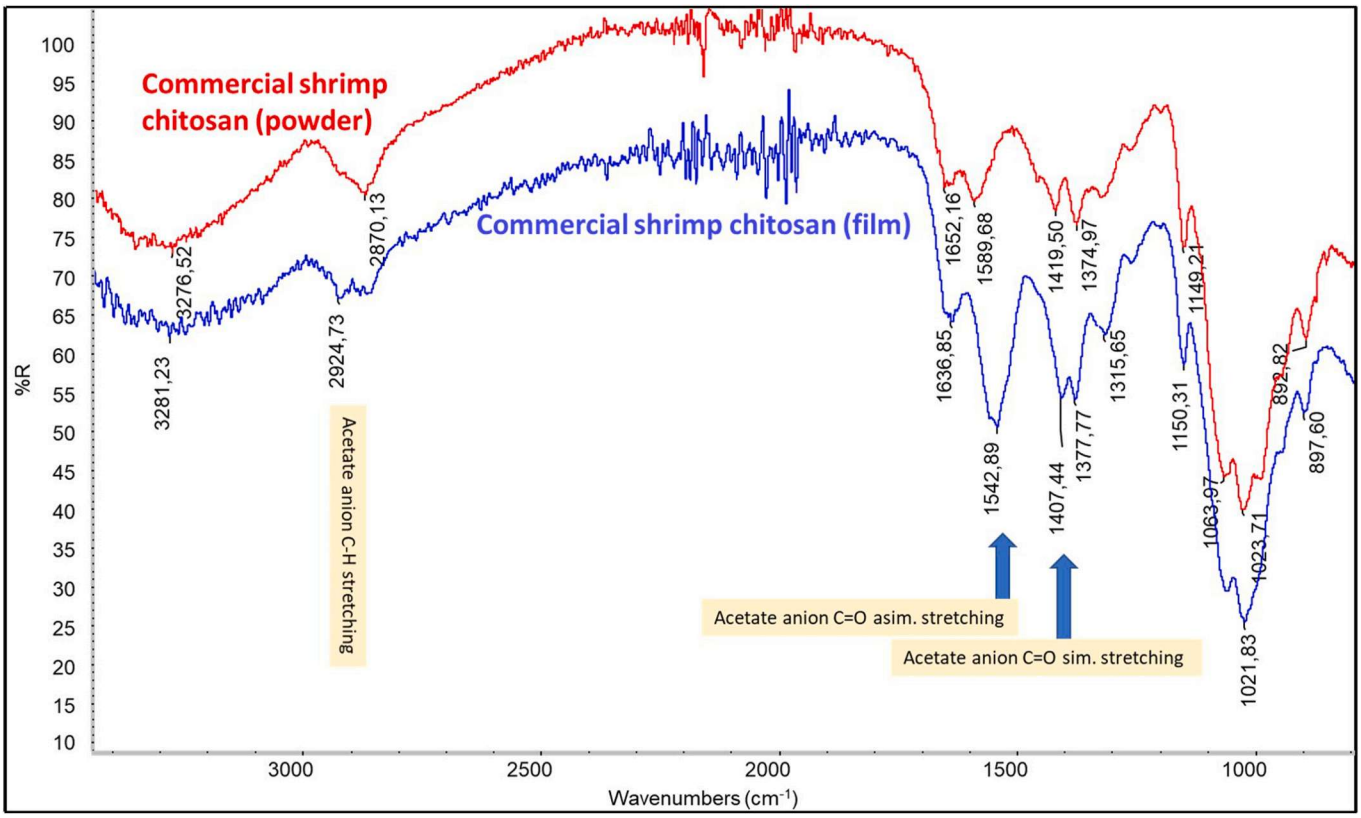

Fig. 6. Infrared spectrum of commercial chitosan sample in powder compared with the spectrum of films produced in 1 % acetic acid.

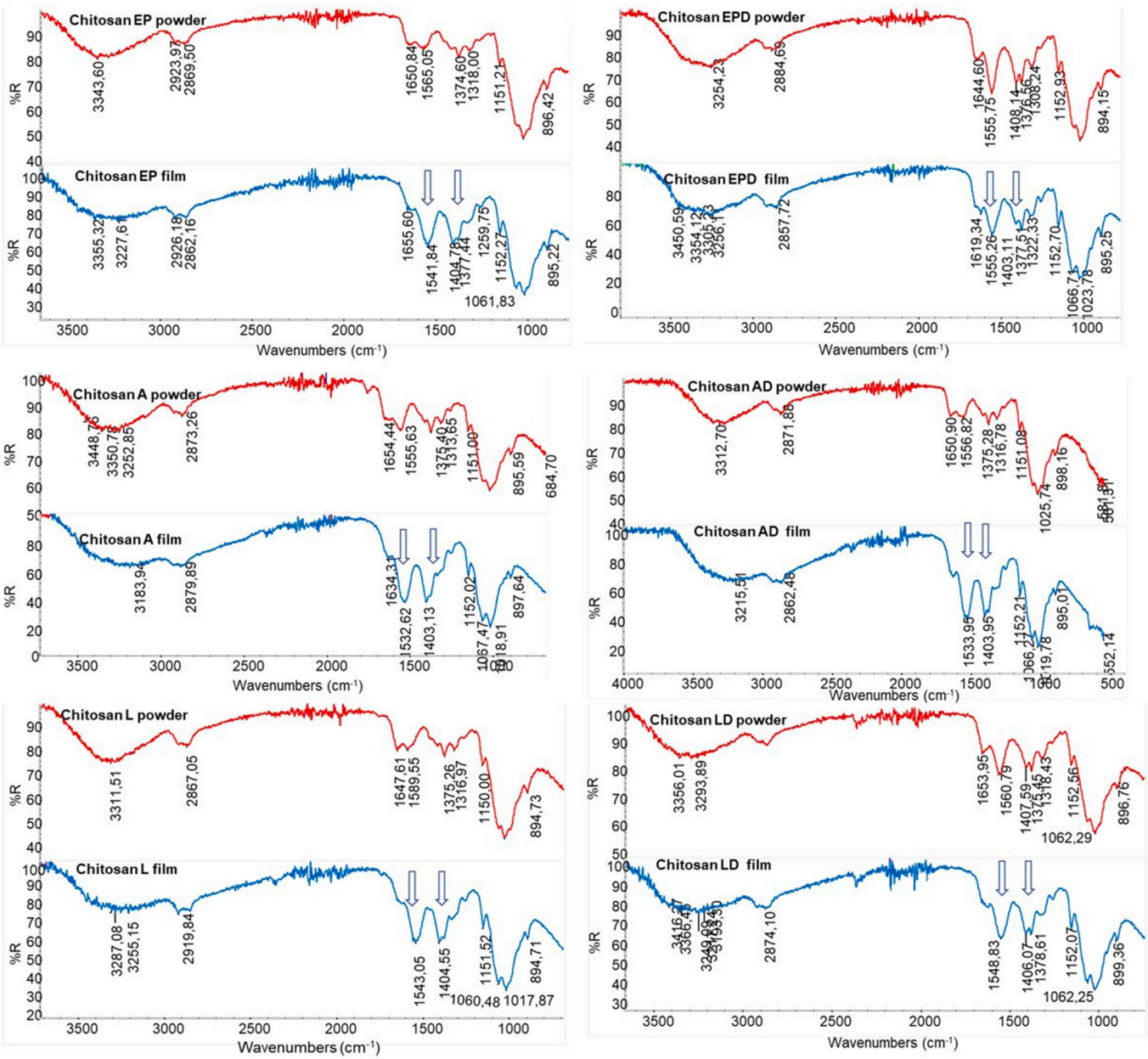

Fig. 7. ATR infrared spectra of chitosan films from HI compared with the respective chitosan samples in powder.

extrusion) and for this reason chitosan and chitin are mainly explored to produce barrier coatings [85,86] for innovative cellulosic based products [87] or bioplastic based films [88].

4. Discussion

In order to correlate the information collected about chemical structure of chitosan samples with the mechanical results related to the chitosan films, the values of the different mechanical properties were reported as a function of the R_{AC} (Fig. 10). Interestingly, it was found that the elastic Modulus tends to increase by increasing the RAC, probably because of the capacity of the acetyl groups to create better interactions between polymeric chains than the charged groups. The acetate anion, in fact, is interacting with a single -NH^{3+} charged positively group and does not contribute to improve intermacromolecular interactions between different chains. On the other hand, accordingly, a higher R_{AC} results generally in a lower elongation at break, despite the films with a less blocky copolymeric distribution as resulting from TGA tests showed a good deformability before breakage. Interestingly, the tensile strength shows values very similar for the different samples, with the exception of CHITO- L and CHITO-SH, that showed the highest values. Interestingly, these samples are exactly those resulting less blocky in repeating units' distribution from our previous investigations.

Thus, the improvement of tensile strength, as well as the improvement of elongation at break, can be attributed to the beneficial effect of having a random distribution of N-acetyl glucosamine units in the chitosan structure, with an optimized balance of effective intermacromolecular interactions and material deformability (Fig. 11). In fact, the latter can reduce the protonated chitosan system intrinsic fragility.

5. Conclusions

Chitin samples obtained from black soldier fly rearing, representing various developmental stages (larvae, pupal exuviae, and adults), were characterized by ATR-IR spectroscopy to determine the R_{AC} ratio, which

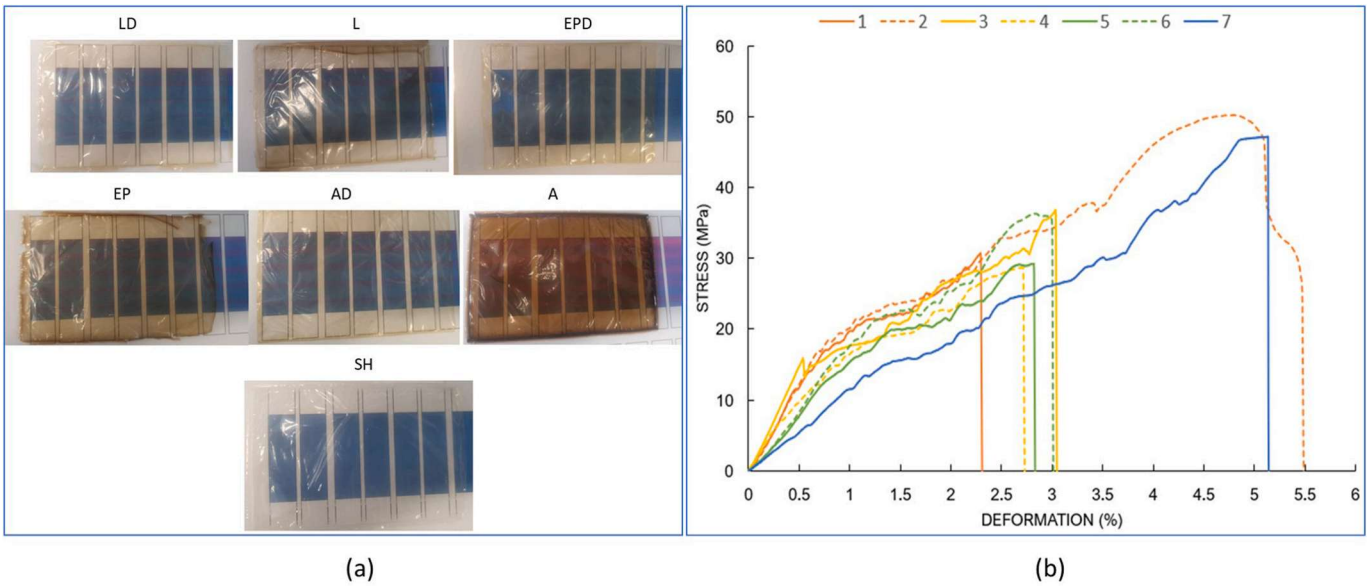

Fig. 8. (a) Films obtained from chitosan of HI, compared with the film obtained in the same conditions using a commercial chitosan from shrimps (SH); (b) representative stress vs deformation trends recorded for chitosan films.

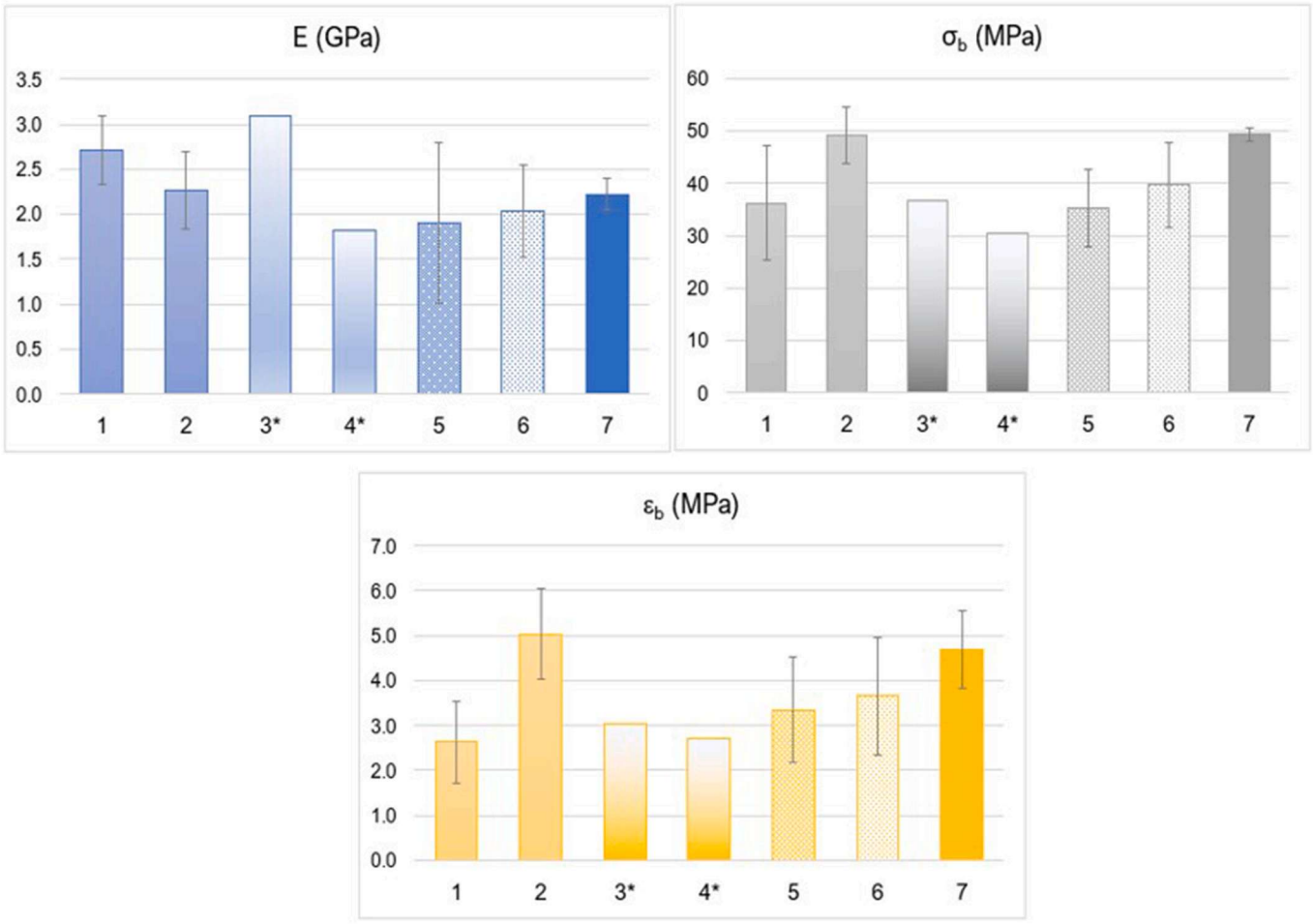

Fig. 9. Mechanical properties of chitosan films: (a) Elastic Modulus obtained by DMTA experiments; (b) tensile strength obtained by tensile tests; (c) elongation at break obtained by tensile tests.

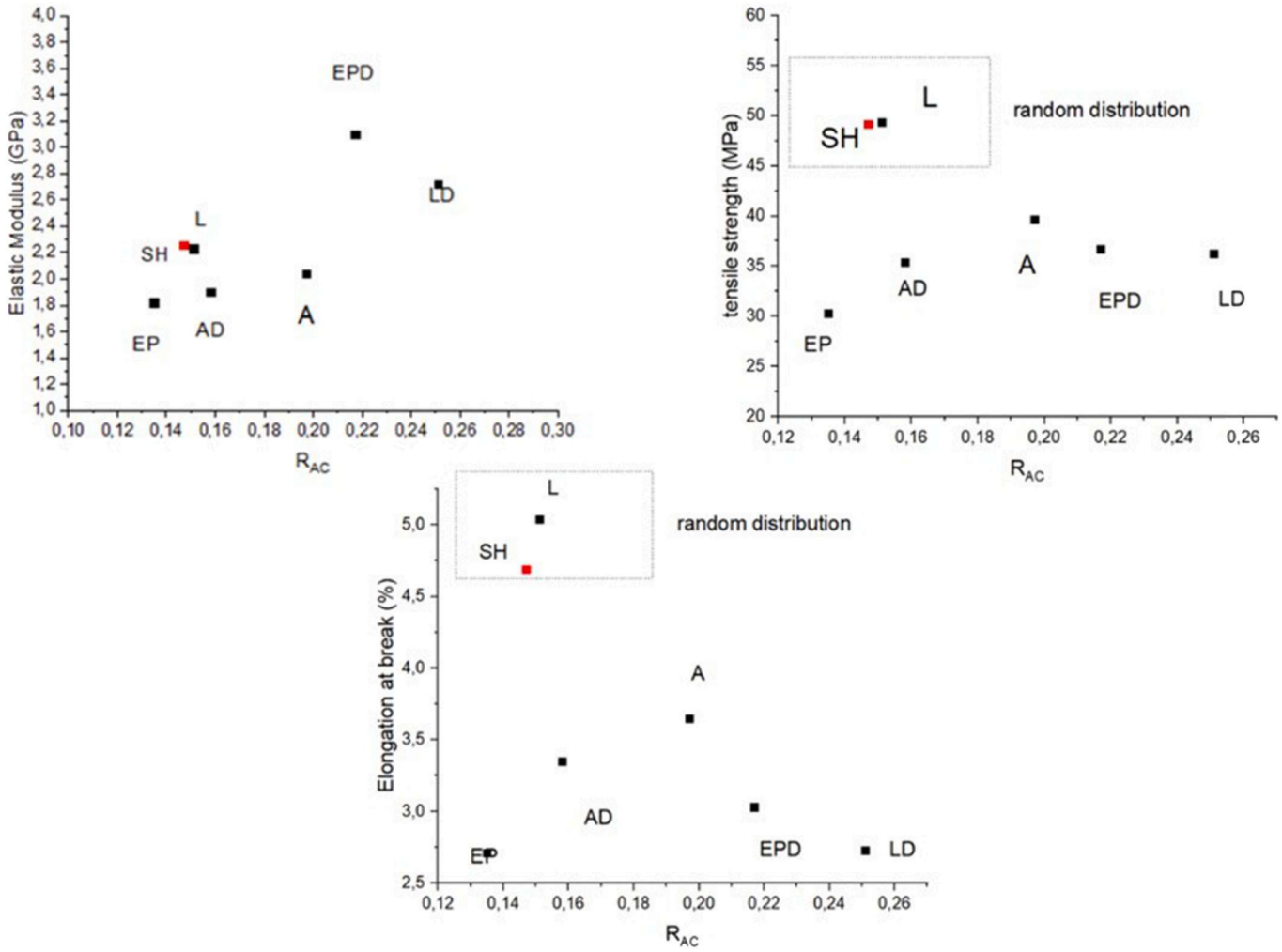

Fig. 10. Correlation between mechanical properties and R_{AC}: (a) Elastic Modulus; (b) Elongation at break; (c) tensile strength.

is associated with the degree of acetylation of the polymers. This parameter was found to be greater than 0.30 for chitin samples and lower than 0.25 for chitosan samples. Notably, chitin derived from adult insects exhibited the highest degree of acetylation, with values exceeding those observed in commercial chitin derived from shrimp and fungi. Discoloured samples showed a lower degree of acetylation compared to their non-discoloured counterparts. As for the chitosan samples, those obtained from larvae and pupal exuviae were the most deacetylated, resembling commercial shrimp-derived chitosan. Overall, the deacetylation process appeared to be less effective in the discoloured samples.

Thermogravimetric analysis performed under a nitrogen atmosphere revealed distinct thermal behaviors between chitin and chitosan samples. In chitosan, an additional mass loss was observed, the magnitude of which decreased with increasing R_{AC} values. Furthermore, it was concluded that chitosan samples obtained through heterogeneous deacetylation predominantly exhibited a blocky distribution of acetamide groups. An exception was observed in the chitosan derived from larvae, as well as in the commercial shrimp-derived chitosan, both of which appeared to possess a primarily homogeneous, random distribution of acetamide groups. along the polymer backboneThe mechanical properties of films cast from acidic aqueous solutions of chitosan exhibited values characteristic of brittle materials. The elastic modulus, determined by DMTA analysis, increased with rising R_{AC} values, while the relationship between R_{AC} and both elongation at break and tensile strength appeared more complex and non-linear.The highest values of tensile strength and elongation at break were recorded for films prepared from chitin derived from *Hermetia illucens* larvae and from commercial chitosan obtained from shrimp. Notably, these samples exhibited a homogeneous distribution of acetyl groups, as indicated by TGA results, suggesting that acetyl group distribution may play a key role in determining the mechanical performance of chitosan-based films.

CRediT authorship contribution statement

Maria-Beatrice Coltelli: Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Vito Gigante:** Writing – original draft, Visualization, Methodology, Data curation. **Luca Panariello:** Methodology, Investigation. **Laura Aliotta:** Writing – review & editing, Validation, Formal analysis. **Carmen Scieuzo:** Writing – review & editing, Methodology. **Patrizia Falabella:** Writing – review & editing, Validation, Supervision, Resources. **Andrea Lazzeri:** Supervision.

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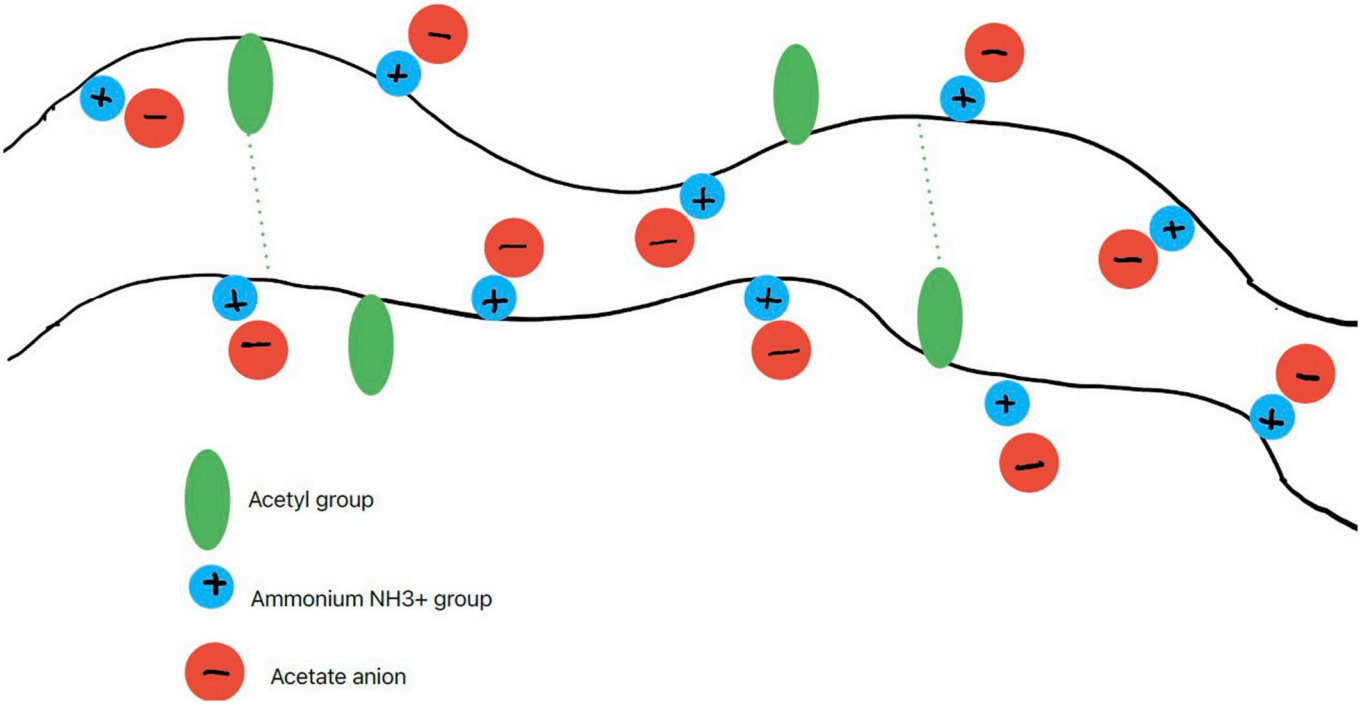

Fig. 11. Schematic chemical structure regarding interactions between two chitosan macromolecules fragments, represented by black lines, in films.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria-Beatrice Coltelli reports financial support was provided by Partnership for Research and Innovation in the Mediterranean Area (PRIMA). Co-authors involved in the development of chitosan from insects in the framework of X-Flies, spin-off company of the University of Basilicata (one of the indicated affiliations) and other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

HI = *Hermetia illucens*
ATR = Attenuated Total Reflectance IR = Infrared
TGA = Thermogravimetric Analysis DTG = Derivative Thermo Gravimetry
DMTA = Dynamical Mechanical Thermal Analysis DSC = Differential Scanning calorimetry
SEM = Scanning Electron Microscopy CHITI = sample consisting of chitin CHITO = sample consisting of chitosan
L = suffix indicating sample obtained from HI larvae
EP = suffix indicating sample obtained from HI pupal exuviae A = suffix indicating sample obtained from HI adult insects
LD = suffix indicating sample obtained from HI larvae and discoloured
EPD = suffix indicating sample obtained from HI pupal exuviae and discoloured AD = suffix indicating sample obtained from HI adult insects and discoloured
SH = suffix indicating a commercial reference sample obtained from shrimps FUN = suffix indicating a commercial reference sample obtained from fungi

Data availability

Data will be made available on request.

References

[1] N.D. Sanandiya, C. Ottenheim, J.W. Phua, A. Caligiani, S. Dritsas, J.G. Fernandez, Circular manufacturing of chitinous bio-composites via bioconversion of urban refuse, Sci. Rep. 10 (1) (2020) 1–8.
[2] R. Kumari, M. Kumar, V. Vivekanand, N. Pareek, Chitin biorefinery: a narrative and prophecy of crustacean shell waste sustainable transformation into bioactives and renewable energy, Renew. Sustain. Energy Rev. 84 (2023).
[3] M. Ul-Islam, K.F. Alabbosh, S. Manan, S. Khan, F. Ahmad, M.W. Ullah, Chitosan-based nanostructured biomaterials: synthesis, properties, and biomedical applications, Adv. Indus. Eng. Polymer Res. 7 (1) (2024) 79–99.
[4] K. Suyeon, Competitive biological activities of chitosan and its derivatives: antimicrobial, antioxidant, anticancer, and anti-inflammatory activities, Int. J. Polym. Sci. (2018) 1708172, 13.
[5] M. Flórez, E. Guerra-Rodríguez, P. Cazón, M. Vázque, Chitosan for food packaging: recent advances in active and intelligent films, Food Hydrocoll. 124 (Part B) (2022) 107328.
[6] K. Stefanowska, M. Woźniak, R. Dobrucka, I. Ratajczak, Chitosan with natural additives as a potential food packaging, Materials 16 (4) (2023) 1579.
[7] P.A. Mistry, M.N. Konar, S. Latha, U. Chadha, P. Bhardwaj, T.K. Eticha, Chitosan superabsorbent biopolymers in sanitary and hygiene applications, Int. J. Polym. Sci. 2023 (2023).
[8] M. Coltelli, P. Morganti, V. Castelvetro, A. Lazzeri, S. Danti, B. Benjelloun-mlayah, et al., Chitin Nanofibril-Nanolignin Complexes as Carriers of Functional Molecules for Skin Contact Applications, vols. 1–23, 2022.
[9] P. Morganti, G. Morganti, M.B. Coltelli, Natural polymers and cosmeceuticals for a healthy and circular life: the examples of chitin, chitosan, and lignin, Cosmetics 10 (2) (2023) 1–14.
[10] I. Aranaz, N. Acosta, C. Civera, B. Elorza, J. Mingo, C. Castro, et al., Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives, Polymers 10 (2) (2018).
[11] M. Ul-Islam, K. Fahad Alabbosh, S. Manan, S. Khan, F. Ahmad, M. Wajid Ullah, Chitosan-based nanostructured biomaterials: synthesis, properties, and biomedical applications, Adv. Indus. Eng. Polymer Res. 7 (2024) 79–99.

[12] M. Triunfo, E. Tafi, A. Guarnieri, C. Scieuzo, T. Hahn, S. Zibek, et al., Insect chitin-based nanomaterials for innovative cosmetics and cosmeceuticals, Cosmetics 8 (2021) 40.

[13] S. Manna, A. Seth, P. Gupta, G. Nandi, R. Dutta, S. Jana, et al., Chitosan derivatives as carriers for drug delivery and biomedical applications, ACS Biomater. Sci. Eng. 9 (5) (2023) 2181–2202.

[14] K. Piekarska, M. Sikora, M. Owczarek, J. Jóźwik-Pruska, M. Wiśniewska-Wrona, Chitin and chitosan as polymers of the future—obtaining, modification, life cycle assessment and main directions of application, Polymers 15 (4) (2023).

[15] I. Aranaz, A.R. Alcántara, M.C. Civera, C. Arias, B. Elorza, A.H. Caballero, et al., Chitosan: an overview of its properties and applications, Polymers 13 (19) (2021).

[16] M. Triunfo, A. Guarnieri, D. Ianniciello, L. Coviello, A. Vitti, M. Nuzzaci, et al., Hermetia illucens, an innovative and sustainable source of chitosan-based coating for postharvest preservation of strawberries, iScience 26 (2023) 108576.

[17] M. Triunfo, A. Guarnieri, D. Ianniciello, M.B. Coltelli, R. Salvia, C. Scieuzo, et al., A comprehensive characterization of Hermetia illucens derived chitosan produced through homogeneous deacetylation, Int. J. Biol. Macromol. 271 (P2) (2024) 132669.

[18] E.C. Achinivu, J.L. Shamshina, R.D. Rogers, Chitin extracted from various biomass sources: it's not the same, Fluid Phase Equilib. 552 (2022) 113286.

[19] M.B. Coltelli, L. Panariello, A. Vannozzi, V. Gigante, A. Gagliardini, P. Morganti, et al., Chitin and its derivatives: nanostructured materials from different marine and terrestrial sources, Chem. Eng. Trans. 93 (April) (2022) 295–300.

[20] J. Ma, Y. Faqir, C. Tan, G. Khaliq, Terrestrial insects as a promising source of chitosan and recent developments in its application for various industries, Food Chem. 373 (2021) 131407.

[21] B.T. Iber, N.A. Kasan, D. Torsabo, J.W. Omuwa, A review of various sources of chitin and chitosan in nature, J. Renew. Mater. 10 (4) (2022) 1097–1123.

[22] V. Grifoll, P. Bravo, M.N. Pérez, M. Pérez-Clavijo, M. García-Castrillo, A. Larrañaga, et al., Environmental sustainability and physicochemical property screening of chitin and chitin-glucan from 22 fungal species, ACS Sustain. Chem. Eng. 12 (20) (2024) 7869–7881.

[23] I. Younes, M. Rinaudo, Chitin and chitosan preparation from marine sources. Structure, properties and applications, Mar. Drugs 13 (3) (2015) 1133–1174.

[24] Y. Alfiko, D. Xie, R.T. Astuti, J. Wong, L. Wang, Insects as a feed ingredient for fish culture: status and trends, Aquacul. Fish. 7 (2) (2022) 166–178.

[25] M. Mannaa, A. Mansour, I. Park, D.W. Lee, Y.S. Seo, Insect-based agri-food waste valorization: agricultural applications and roles of insect gut microbiota, Environ. Sci. Ecotechnol. 17 (2024) 100287.

[26] A. Mannucci, L. Panariello, L. Abenaim, M.B. Coltelli, A. Ranieri, B. Conti, et al., From food waste to functional biopolymers: characterization of chitin and chitosan produced from prepupae of black soldier fly reared with different food waste-based diets, Foods 13 (2) (2024).

[27] J. Suckling, A. Druckman, R. Small, F. Cecelja, M. Bussemaker, Supply chain optimization and analysis of Hermetia illucens (Black soldier fly) bioconversion of surplus foodstuffs, J. Clean. Prod. 321 (August) (2021) 128711.

[28] S. Amine, A. Montembault, M. Fumagalli, A. Osorio-Madrazo, L. David, Controlled polyelectrolyte association of chitosan and carboxylated nano-fibrillated cellulose by desalting, Polymers 13 (12) (2021).

[29] F.E. Bentley, R. Passieux, L. David, A. Osorio-Madrazo, Pure chitosan biomedical textile fibers from mixtures of Low- and high-molecular weight bidisperse polymer solutions: processing and understanding of microstructure-mechanical properties' relationship, Int. J. Mol. Sci. 23 (9) (2022).

[30] I. Younes, S. Sellimi, M. Rinaudo, K. Jellouli, M. Nasri, Influence of acetylation degree and molecular weight of homogeneous chitosans on antibacterial and antifungal activities, Int. J. Food Microbiol. 185 (2014) 57–63.

[31] E.I. Akpan, O.P. Gbenebor, S.O. Adeosun, O. Cletus, Solubility, Degree of Acetylation, and Distribution of Acetyl Groups in Chitosan, INC, 2020.

[32] V. Zargar, M. Asghari, A. Dashti, A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications, ChemBioEng Rev. 2 (3) (2015) 204–226.

[33] M.X. Weinhold, J.C.M. Sauvageau, J. Kumirska, J. Thöming, Studies on acetylation patterns of different chitosan preparations, Carbohydr. Polym. 78 (4) (2009) 678–684.

[34] Q. Luo, Y. Wang, Q. Han, L. Ji, H. Zhang, Z. Fei, Y. Wang, Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects, Carbohydr. Polym. 209 (2019) 266–275.

[35] N.H. Marei, E.A. El-Samie, T. Salah, G.R. Saad, A.H.M. Elwahy, Alternative processes to produce chitin, chitosan, and their oligomers isolation and characterization of chitosan from different local insects in Egypt, Int. J. Biol. Macromol. 82 (2016) 871–877.

[36] J. Ma, C. Xin, C. Tan, Preparation, physicochemical and pharmaceutical characterization of chitosan from Catharsius molossus residue, Int. J. Biol. Macromol. 80 (2015) 547–556.

[37] P. Morganti, R.A.A. Muzzarelli, C. Muzzarelli, Multifunctional use of innovative chitin nanofibrils for skin care, J. Appl. Cosmetol. 24 (3) (2006) 105–114.

[38] N. Özel, M. Elibol, A review on the potential uses of deep eutectic solvents in chitin and chitosan related processes, Carbohydr. Polym. 262 (2021) 117942.

[39] W.M. Argüelles-Monal, J. Lizardi-Mendoza, D. Fernández-Quiroz, M.T. Recillas-Mota, M. Montiel-Herrera, Chitosan derivatives: introducing new functionalities with a controlled molecular architecture for innovative materials, Polymers 10 (3) (2018) 342.

[40] E.B. Holm, Elise Borgen Holmås Master ' S Thesis Chitin- B -chitosan Diblocks: Preparation and Characterization, 2022.

[41] N. Azmi, S.S.M. Lock, N.T. Berghuis, A. Sarwono, N.L. Zahra, A. Rahman, et al., Influence of synthesis approach and formulation on the physicochemical properties of chitin and chitosan from black soldier fly, Results Eng. 23 (2024) 102401.

[42] C. Song, H. Yu, M. Zhang, Y. Yang, G. Zhang, Physicochemical properties and antioxidant activity of chitosan from the blowfly Chrysomya megacephala larvae, Int. J. Biol. Macromol. 60 (2013) 347–354.

[43] Q. Luo, Y. Wang, Q. Han, L. Ji, H. Zhang, Z. Fei, et al., Comparison of the physicochemical, rheological, and morphologic properties of chitosan from four insects, Carbohydr. Polym. 209 (2019) 266–275.

[44] Y. Bolat, Ş. Bilgin, A. Günlü, L. Izci, S.B. Koca, S. Çetinkaya, et al., Chitin-chitosan yield of freshwater crab (Potamon potamios, olivier 1804) shell, Pak. Vet. J. 30 (4) (2010) 227–231.

[45] M.S. Hossain, A. Iqbal, Production and characterization of chitosan from shrimp waste, J. Bangladesh Agric. Univ. 12 (1) (2014) 153–160.

[46] R.J.S. de Castro, A. Ohara, J.G. dos Santos Aguilar, M.A.F. Domingues, Nutritional, functional and biological properties of insect proteins: processes for obtaining, consumption and future challenges, Trends Food Sci. Technol. 76 (2018) 82–89.

[47] I. Younes, M. Rinaudo, Chitin and chitosan preparation from marine sources. Structure, properties and applications, Mar. Drugs 13 (3) (2015) 1133–1174.

[48] J.G. Monter-Miranda, J.M. Tirado-Gallegos, P.B. Zamudio-Flores, C. Rios-Velasco, J. de J. Ornelas-Paz, R. Salgado-Delgado, et al., Extraction and characterization of phisicochemical, morphological, and structural properties of chitin and chitosan from Brachystola magna (girard), Rev. Mex. Ing. Quim. 15 (3) (2016) 749–761.

[49] S. Islam, M.A.R. Bhuiyan, M.N. Islam, Chitin and chitosan: structure, properties and applications in biomedical engineering, J. Polym. Environ. 25 (3) (2017) 854–866.

[50] R.S.C.M. de Queiroz Antonino, B.R.P. Lia Fook, V.A. de Oliveira Lima, R.Í. de Farias Rached, E.P.N. Lima, R.J. da Silva Lima, et al., Preparation and characterization of chitosan obtained from shells of shrimp (*Litopenaeus vannamei Boone*), Mar. Drugs 15 (5) (2017).

[51] M. Kaya, T. Baran, S. Erdoğan, A. Menteş, M. Aşan Özüsağlam, Y.S. Çakmak, Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata), Mater. Sci. Eng. C 45 (2014) 72–81.

[52] S. Erdogan, M. Kaya, High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper, Int. J. Biol. Macromol. 89 (2016) 118–126.

[53] G. Tan, M. Kaya, A. Tevlek, I. Sargin, T. Baran, Antitumor activity of chitosan from mayfly with comparison to commercially available low, medium and high molecular weight chitosans, Vitro Anim. Cell Dev. Biol. 54 (5) (2018) 366–374.

[54] M.-W. Kim, Y.-S. Song, Y.S. Han, Y.H. Jo, M.H. Choi, Y.-K. Park, et al., Production of chitin and chitosan from the exoskeleton of adult two-spotted field crickets (gryllus bimaculatus), Entomol. Res. 47 (5) (2017) 279–285.

[55] A.T. Paulino, J.I. Simionato, J.C. Garcia, J. Nozaki, Characterization of chitosan and chitin produced from silkworm crysalides, Carbohydr. Polym. 64 (1) (2006) 98–103.

[56] M. Iñiguez-Moreno, B. Santiesteban-Romero, E.M. Melchor-Martínez, R. Parra-Saldívar, R.B. González-González, Valorization of fishery industry waste: Chitosan extraction and its application in the industry, MethodsX 13 (August) (2024).

[57] H. Tiernan, B. Byrne, S.G. Kazarian, ATR-FTIR spectroscopy and spectroscopic imaging for the analysis of biopharmaceuticals, Spectrochim. Acta Mol. Biomol. Spectrosc. 241 (2020) 118636.

[58] D.I. Sánchez-Machado, J. López-Cervantes, A.A. Escárcega-Galaz, O.N. Campas-Baypoli, D.M. Martínez-Ibarra, S. Rascón-León, Measurement of the degree of deacetylation in chitosan films by FTIR, ^{1}H NMR and UV spectrophotometry, MethodsX 12 (2014) 102583.

[59] H.U. Rehman, S. Cord-Landwehr, V. Shapaval, S. Dzurendova, A. Kohler, B. M. Moerschbacher, B. Zimmermann, High-throughput vibrational spectroscopy methods for determination of degree of acetylation for chitin and chitosan, Carbohydr. Polym. 302 (2023) 120428.

[60] S. Erdogan, M. Kaya, High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper, Int. J. Biol. Macromol. 89 (2016) 118–126.

[61] M. Kaya, T. Baran, S. Erdołan, A. Menteş, M. Aşan Özüsałlam, Y.S. Çakmak, Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (Leptinotarsa decemlineata), Mater. Sci. Eng. C. 45 (2014) 72–81.

[62] J. Kumirska, M. Czerwicka, Z. Kaczyński, A. Bychowska, K. Brzozowski, J. Thöming, P. Stepnowski, Application of spectroscopic methods for structural analysis of chitin and chitosan, Mar. Drugs 8 (2010) 1567–1636.

[63] H. Percentage, C.D.T. Cells, Attenuated Total Reflectance-Fourier Transform Infrared, 2022 (ll).

[64] J. Brugnerotto, J. Lizardi, F.M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, An infrared investigation in relation with chitin and chitosan characterization, Polymer 42 (8) (2001) 3569–3580.

[65] M. Triunfo, E. Tafi, A. Guarnieri, R. Salvia, C. Scieuzo, T. Hahn, et al., Characterization of chitin and chitosan derived from Hermetia illucens, a further step in a circular economy process, Sci. Rep. 12 (1) (2022) 1–17.

[66] J. Zawadzki, H. Kaczmarek, Thermal treatment of chitosan in various conditions, Carbohydr. Polym. 80 (2) (2010) 394–400.

[67] H.F.G. Barbosa, D.S. Francisco, A.P.G. Ferreira, É.T.G. Cavalheiro, A new look towards the thermal decomposition of chitins and chitosans with different degrees of deacetylation by coupled TG-FTIR, Carbohydr. Polym. 225 (June) (2019) 115232.

[68] L.S. Guinesi, É.T.G. Cavalheiro, The use of DSC curves to determine the acetylation degree of chitin/chitosan samples, Thermochim. Acta 444 (2) (2006) 128–133.

[69] T. Wanjun, W. Cunxin, C. Donghua, Kinetic studies on the pyrolysis of chitin and chitosan, Polym. Degrad. Stabil. 87 (3) (2005) 389–394.
[70] E. Melro, F.E. Antunes, G.J. da Silva, I. Cruz, Changing acidic dissolution conditions, Polymers 1–12 (2021).
[71] A. De Masi, I. Tonazzini, C. Masciullo, R. Mezzena, F. Chiellini, D. Puppi, et al., Chitosan films for regenerative medicine: fabrication methods and mechanical characterization of nanostructured chitosan films, Biophys. Rev. 11 (5) (2019) 807–815.
[72] L. Panariello, M.-B. Coltelli, M. Buchignani, A. Lazzeri, Chitosan and nano-structured chitin for biobased anti-microbial treatments onto cellulose based materials, Eur. Polym. J. 113 (2019).
[73] T. Hahn, A. Roth, R. Ji, E. Schmitt, S. Zibek, Chitosan production with larval exoskeletons derived from the insect protein production, J. Biotechnol. 310 (2020) 62–67. December 2019.
[74] M.R. Kasaai, A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy, Carbohydr. Polym. 71 (4) (2008) 497–508.
[75] S.H. Lim, S.M. Hudson, Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group, Carbohydr. Res. 339 (2) (2004) 313–319.
[76] D. Zvezdova, V. Georgieva, L. Vlaev, Non–Isothermal Kinetics of Degradation of Chitin and Chitosan, 2011, pp. 13–18.
[77] S. Arora, S. Lal, S. Kumar, M. Kumar, M. Kumar, Comparative degradation kinetic studies of three biopolymers : chitin , chitosan and cellulose, Arch. Appl. Sci. Res. 3 (3) (2011) 188–201.
[78] I. Corazzari, R. Nisticò, F. Turci, M.G. Faga, F. Franzoso, S. Tabasso, et al., Advanced physico-chemical characterization of chitosan by means of TGA coupled on-line with FTIR and GCMS: thermal degradation and water adsorption capacity, Polym. Degrad. Stabil. 112 (2015) 1–9.
[79] Y.S. Nam, W.H. Park, D. Ihm, S.M. Hudson, Effect of the degree of deacetylation on the thermal decomposition of chitin and chitosan nanofibers, Carbohydr. Polym. 80 (1) (2010) 291–295.
[80] J.M.F. Pavoni, C.L. Luchese, I.C. Tessaro, Impact of acid type for chitosan dissolution on the characteristics and biodegradability of cornstarch/chitosan based films, Int. J. Biol. Macromol. 138 (2019) 693–703.
[81] K. Ito, H.J. Bernstein, The vibrational spectra of the formate, acetate, and oxalate ions, Can. J. Chem. 34 (2) (1956) 170–178.
[82] J.F. Rubilar, R.M.S. Cruz, H.D. Silva, A.A. Vicente, I. Khmelinskii, M.C. Vieira, Physico-mechanical properties of chitosan films with carvacrol and grape seed extract, J. Food Eng. 115 (4) (2013) 466–474.
[83] C. Madeleine-Perdrillat, T. Karbowiak, F. Debeaufort, L. Delmotte, C. Vaulot, D. Champion, Effect of hydration on molecular dynamics and structure in chitosan films, Food Hydrocoll. 61 (2016) 57–65.
[84] X. Ma, C. Qiao, X. Wang, J. Yao, J. Xu, Structural characterization and properties of polyols plasticized chitosan films, Int. J. Biol. Macromol. 135 (2019) 240–245.
[85] S. Roy, K.L. Goh, C. Verma, B. Dasgupta Ghosh, K. Sharma, P.K. Maji, A facile method for processing durable and sustainable superhydrophobic chitosan-based coatings derived from waste crab shell, ACS Sustain. Chem. Eng. 10 (14) (2022) 4694–4704.
[86] Z. Cheng, J. Li, M. Su, N. Xiao, L. Zhong, X. Zhang, et al., High-barrier oxidized cellulose nanofibril/chitosan coating for functional food packaging materials, ACS Appl. Polym. Mater. 6 (5) (2024) 2877–2888.
[87] L. Panariello, M.B. Coltelli, S. Giangrandi, M.C. Garrigós, A. Hadrich, A. Lazzeri, et al., Influence of functional bio-based coatings including chitin nanofibrils or polyphenols on mechanical properties of paper tissues, Polymers 14 (11) (2022) 1–20.
[88] L. Panariello, M.B. Coltelli, A. Hadrich, F. Braca, S. Fiori, A. Haviv, et al., Antimicrobial and gas barrier crustaceans and fungal chitin-based coatings on biodegradable bioplastic films, Polymers 14 (23) (2022) 5211.