Biodegradability study of active *Quercus* extract chitosan biopolymer films in different soil types

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Abstract

One of the recent trends within the circular economy is the development of materials derived from food processing waste and their utility as an alternative to plastic packaging. In this context, the study aims to evaluate biological causes of deterioration or degradation of chitosan-based films with and without incorporated natural *Quercus* extract in three different types of soils (industrial compost, commercial garden soil, and soil from a vineyard). Degradation and active properties deterioration was followed by measurement of the loss of mass of tested active films for 14 days, and it was accompanied by other analytical techniques such as measurement of polyphenolic content, FT-IR analysis, and SEM examination of the packaging morphology. The results showed that chitosan-based film properties deteriorate in less than 3 days followed by biodegradation in all tested soils after 14 days. Films with incorporated *Quercus* extract undergo deterioration of active properties in compost and garden

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soil in 6 days, while the fractionation and degradation process has not been complete in the vineyard soil during the 14 days. Furthermore, it has also been revealed that the addition of water to the soil decreased the rate of active chitosan film biodegradation in the terrestrial environment.

Keywords: Fractionation and biodegradation, biopolymer; chitosan-based films; active packaging material; *Quercus* extract; terrestrial environment.

Abbreviation list

AA	antioxidant activity
AW	soils with the addition of water
CH	chitosan-based film
CHQ	chitosan-based film with incorporated *Quercus* extract
DA	dehydrogenase activity
FC	Folin-Ciocalteu reagent
GAE	gallic acid mass equivalent
MC	moisture content
NMC	soils with natural moisture content
SMC	soil moisture content
SOM	soil organic matter
TOC	total organic carbon
TPC	total polyphenolic content
TSM	total soluble matter
TTC	triphenyltetrazolium chloride
TTP	triphenylformazane
WVP	water vapour permeability
WVT	water vapour transmittance

1 Introduction

The term ‘plastic’ describes a wide range of materials, which can be used for various purposes. A noteworthy amount of plastics produced in the EU is used for packaging (39.9.% in the year 2018) (Kumar et al., 2017; PlasticsEurope, 2019). Furthermore, among plastic packaging, food packaging has the shortest lifetime as the food shelf life determines its maximum lifetime. In Slovenia, between 40% and 50% of waste packaging is recycled while the rest is incinerated, ends up in landfills, or is not collected due to littering and unauthorised dumping (Kranjc and Pungerčar, 2019; PlasticsEurope, 2019). Since the majority of plastic materials are hardly degradable under natural conditions, they accumulate and persist in the environment for years and decades, and thus constitute a major concern regarding plastic pollution (Yuan et al., 2020). Besides, the current problem presented by plastics in the environment will increase for years to come as large pieces of plastic break down into smaller pieces called microplastics (Kalčíková et al., 2020).

As conventional plastic packaging materials are based on petroleum, depletion of fossil fuels will limit their future production. Although the future rise of biodegradable plastics can ease these problems, newly developed materials should contribute to the goal of a circular economy in which plastics derive from and are converted back to biomass. World Economic Forum placed the bio-based plastics for a circular economy as number 1 on the top 10 emerging technologies for 2019 (WEF, 2019). In respect to this, more environmentally friendly alternatives for plastic packaging are extensively studied (Gabor Naiaretti and Tita, 2012; Miteluț et al., 2015; Moschakis and Biliaderis, 2017).

Biopolymers are widely available from renewable sources such as biomass and industry side streams and exhibit good mechanical properties, and most importantly, their inherent biodegradability in the environment. However, the options currently available – mostly made from corn, sugar cane, or waste fats, and oils – generally lack the mechanical strength and

visual characteristics of the standard kinds. The utilization of chitin/chitosan as a matrix for bio-based plastic packaging has been intensively investigated in this respect (Cazón et al., 2017; Gabor Naiaretti and Tita, 2012; Rinaudo, 2006; Wang et al., 2018; Bajić et al., 2019b).

Chitin is the second most abundant polysaccharide (after cellulose) in nature. It is commonly obtained from waste shrimp or crab shells of the food processing industry and fungal mycelia. Chitosan is a water-soluble derivative of chitin obtained by alkaline N-deacetylation of chitin (Labrie et al., 2001; Rubilar et al., 2013). In 2016, the aquaculture production for crustaceans reached 16 million tonnes (live weight), which leads to a million tons of shell waste (FAO, 2018). Crustacean shells consist of 20–40% protein, 20–50% calcium carbonate and 15–40% chitin. This type of bio-waste can be ground down and the powder used as an animal-feed supplement, bait or fertilizer, as well as in chitin/chitosan production (Borić et al., 2018).

Chitosan itself exhibits antibacterial and antioxidant properties, and therefore, it has the potential to be used as a natural-based food preservative packaging material (Novak et al., 2019). The incorporation of various essential oils and plant extracts such as chestnut extract (Bajić et al., 2020; Kõrge et al., 2020a), hop extract (Bajić et al., 2019a), apple peel polyphenols (Zeng et al., 2018), grape seed extract (Shahbazi, 2017), turmeric extract (Kalaycıoğlu et al., 2017) and thyme extract (Talón et al., 2017) can further enhance antibacterial and antioxidant properties of the films. The ability to form films and coatings with good mechanical properties and selective permeability to CO_2 and O_2 together with before mentioned quantities allow chitosan to fulfil packaging requirements (Novak et al., 2019). Moisture content is related to the mechanical properties and can be controlled by the amount of added plasticizer (Bajić et al., 2020). Nonetheless, chitosan films are highly permeable by water vapour, which restricts their application as packaging in certain food industries, considering that effective control of moisture transfer is a very important property for preservation of food quality (Aider, 2010).

Degradation of chitosan-based products has been thoroughly studied in terms of biomedical application and degradation in the human body (Bagheri-Khoulenjani et al., 2009; Matica et al., 2017), as well as in the aquatic environment (Ratajska et al., 2003). However, the degree and mechanisms of chitosan degradation in the terrestrial environment are not yet fully understood. In the broad spectrum the deterioration can occur by the action of heat, stress, radiation, oxidation, hydrolysis and chemical agents, as well as by biological processes, and biodegradation facilitated by preliminary UV irradiation affects the morphology, molecular weight and surface area of the grafted materials. The extent of biodegradation of chitosan-graft-polymethylmethacrylate (C-g-PMMA) films copolymers showed only 50% weight loss due to biodegradation, which accounts for utilization of the available chitosan by *A. flavus* (Prashanth et al., 2005).

In this context, this study aimed to evaluate the biodegradability rate of active films based on chitosan in various of soils. Used chitosan-based films were enhanced by the addition of *Quercus* extract to increase optical, antioxidative and antibacterial properties as shown in our previous work (Bajić et al., 2019b), confirming its active properties. This active packaging material's biodegradability was tested in industrial compost, commercial garden soil, and vineyard soil. The soil properties in terms of moisture, organic and inorganic matter and the urease and dehydrogenase activity were evaluated. The tested soils were kept at their original moisture content and it was shown impossible to be base-lined because of different structures of soils and their water holding capacities. The chitosan-based film biodegradation was determined by the loss of mass during the 14-day fractionation and biodegradation study, accompanied by investigation of surface morphology by SEM and FT-IR. Moreover, the reduction of the films' polyphenolic content before and after degradation were examined in order to study the degradation kinetics of the *Quercus* extract and its correlation to the chitosan degradation rate and soil moisture content.

2 Materials and methods

2.1 Materials

High molecular weight chitosan (310 to 375 kDa, ≥75% deacetylated), 85% lactic acid, Folin-Ciocalteu reagent and gallic acid were bought at Sigma-Aldrich (Steinheim, Germany), sodium carbonate and glycerol from Merck (Darmstadt, Germany) and Pharmachem Sušnik (Ljubljana, Slovenia), respectively. Triphenyltetrazolium chloride (TTC), triphenylformazane (TTP), and tris(hydroxymethyl)aminomethane (Tris base) buffer were bought from (Sigma-Aldrich, Germany), NH_4^+, acetone and urea from (Sigma-Aldrich, Germany). The *Quercus* (oak) extract was provided by Tanin Sevnica (Sevnica, Slovenia). Excluding lactic acid, all chemicals were of analytical grade. Milli-Q water was used through all the experiments unless stated otherwise.

2.2 Chitosan-based films

Preparation of chitosan-based films proceeded at protocol described elsewhere (Bajić et al., 2019b). Briefly, 1.5 % (w/v) of chitosan and glycerol in concentration 30.0 % (based on the weight of chitosan) were dissolved in 1.0% (v/v) aqueous solution of lactic acid. The mixture was stirred on magnetic stirrer IKA® RCT (IKA, Staufen, Germany) overnight at 1000 rpm and room temperature. The film-forming solution was vacuum-filtered through 4 layers of medical gauze to remove impurities. *Quercus* extract was added in the form of powder in concentration 0.1 % (w/v), homogenized with Ultra-Turrax® T50 homogenizer (IKA, Staufen, Germany) at 6000 rpm for 2 min and left in beaker overnight. The foam was skimmed and the film-forming solution was poured to Petri dishes in the amount of 0.32 mL cm^{-2}. Films were dried in the oven at 40 °C for 48 h. Samples were stored under ambient conditions in the dark (in the box that was put in the drawer) until further analysis (Bajić et al., 2019a, 2019b).

Chitosan-based films without any extract and films with incorporated *Quercus* extract were labelled CH and CHQ, respectively.

2.2.1 Moisture content and total soluble matter

Moisture content (*MC*) and total soluble matter (*TSM*) was determined gravimetrically (Bajić et al., 2019b). The film was cut into rectangular pieces (weight less than 7 mg) and weighted on the analytical scale to get the initial mass (M_1). After being dried in an oven at 105 °C for 24 h, the sample was weighed again to get dry mass (M_2). *MC* was calculated according to Equation 1:

$$MC\ (\%) = (M_1 - M_2)/M_1\ x\ 100\ \% \qquad Eq.\ 1$$

and expressed as the percentage of water content in the films. The samples were then transferred to vials containing 5 mL of water. Films were left soaking for 24 h at room temperature and then dried again for 24 h at 105 °C. *TSM* was calculated by use of Equation 2:

$$TSM\ (\%) = (M_2 - M_3)/M_2\ x\ 100\ \% \qquad Eq.\ 2$$

TSM was expressed as the percentage of total dry matter soluble in water throughout 24 h (Bajić et al., 2019a, 2019b). All experiments were done in triplicates. Results present an average of all six measurements of a film type (Section 3.1).

2.2.2 Total phenolic content

Total polyphenolic content (*TPC*) in chitosan-based films was determined using Folin-Ciocalteu (FC) reagent (Bajić et al., 2019b). Up to 5 mg of the film was weighted into vials. Water was added in the amount based on the weight of the sample to reach final concentration 5 mg mL^{-1}. FC reagent and an aqueous solution of 10 wt% Na_2CO_3 were added in the amount to reach final concentration 10 vol% and 20 vol% based on the volume of water, respectively. Solutions were then incubated for 2 h in dark at room temperature. Absorbance was measured at 765 nm using Synergy™ 2 Multi-Detection Microplate Reader (BioTek, Winooski, USA).

Results were expressed as gallic acid mass equivalent (GAE) per dry mass of film (Rambabu et al., 2019). *TPC* was also monitored for 19 days to confirm that it does not change under normal room conditions and is affected exclusively by degradation in soil. *TPC* was also determined after the degradation of chitosan-based films in soils.

2.2.3 Fourier-transform Infrared Spectroscopy analysis of degradation

Fourier-transform Infrared Spectroscopy (FT-IR) spectra of all samples were recorded. The analysis was carried out at room temperature and wavenumbers from 4000 cm^{-1} to 400 cm^{-1}, with resolution 4 cm^{-1}. Spectrum Two FT-IR (PerkinElmer, Waltham, USA) spectrometer was used. The resulting spectra (Section 3.2.4) present the average of three independent measurements. FT-IR spectra were also recorded for samples during degradation to determine changes in the structure of the chitosan-based films.

2.3 Degradation in soils

The degradation experiment proceeded in three types of soils: in industrial compost obtained from a local waste management facility, in commercial garden soil that was purchased in a local gardening shop and in vineyard soil that was obtained from a private vineyard in the Southeast part of Slovenia (46°43'52.0"N 16°09'14.4"E) in November 2018. Degradation was carried out for one type of soil at the time. 250 g of soil was weighted into each of eight crystallising dishes with a diameter of 14 cm. In two of them, degradation was carried out at natural soil moisture content and in the other two, 2.5 mL of water was added. Four chitosan-based films in size of 3 × 3 cm were put in each crystallising dish, to have 4 pieces of CH in both soils with natural humidity and in soil with the addition of water, and 4 pieces of CHQ in both soils with natural moisture content (NMC) and in soils with the addition of water (AW). Visualization of the degradation experiment is presented in Figure A.1.

All chitosan-based films were then analysed after 3, 7, 10, and 14 days of degradation in soils. Residues of films were carefully removed with tweezers; samples were rinsed with distilled water and dried until constant mass. After each sampling, the soil was gently stirred to keep aerobic conditions. All experiments were done in duplicates.

2.3.1 Degree of biodegradation

The degree of biodegradation was determined gravimetrically. Sample mass (M_{before}) and MC (Section 2.2.1) were determined before degradation. After degradation, the sample was dried at 105 °C for 24 hours and weighed on the analytical scale to obtain mass after degradation (M_{after}). The degree of biodegradation was calculated through Equation 3 and was expressed as a percentage (%):

$$degree\ of\ biodegradation\ (\%) = (1 - M_{after})/(M_{before}\ x\ (1 - MC/100)) \qquad Eq.\ 3$$

2.3.2 Soil moisture content

Approximately 20 g of soil was weighed into a Petri dish to obtain m_{sample} and dried in a laboratory dryer (Kambič, Slovenia) at 105 °C for at least one hour, until a constant mass was reached $m_{dry\ sample}$. Soil moisture content (SMC) was calculated using Equation 4:

$$SMC\ (\%) = (m_{sample} - m_{dry\ sample})/m_{sample}\ \ x\ 100 \qquad Eq.\ 4$$

SMC was expressed as the percentage (%) of water per total mass of soil.

2.3.3 Soil organic matter

Firstly, the samples were dried at 105 °C until a constant mass was reached, then bigger particles were crushed in a mortar. Total organic carbon (TOC) was measured with Dorhmann DC-190 TOC (Emerson, St. Louis, USA). Approximately 0.3 mg of sample was weighted and injected onto a platinum catalyst at 800 °C. Synthetic air (200 mL min^{-1}) was used as a carrier gas. CO_2 emissions were detected by NDIR detector. Na_2CO_3 was used as a standard. Analyses

were repeated at least twice. Soil organic matter (*SOM*) was calculated based on Equation 5, proposed by (Broadbent 1953):

$$SOM\ (\%) = TOC\ (\%)\ x\ 1.9 \qquad Eq.\ 5$$

SOM presents the percentage (%) of organic matter in dry soil matter.

2.3.4 Urease activity

The activity of ureases was determined spectrophotometrically by analysing produced ammonia from the hydrolysis of urea. 5 g of soil was weighted into 20 mL Erlenmeyer flasks and 2.5 mL of 0.08 M aqueous solution of urea was added (2.5 mL of water to control). Erlenmeyer flasks were sealed and incubated at 37 °C for 2 h. Hydrolysis was stopped with 50 mL of 1.0 M KCl and 0.01 M HCl. Samples were then mixed on a magnetic stirrer for 30 min. 2.5 mL of urea was added to the control sample just before adding KCl/HCl mixture. Mixtures were then filtered in a dark room and diluted 10 fold. The concentration of ammonium was determined spectrophotometrically according to ISO 7150–1 at 655 nm with spectrophotometer Cary 50 Probe UV-VIS (Varian Australia Pty. Ltd., Mulgrave, Australia).

2.3.5 Dehydrogenase activity

Dehydrogenase activity was measured according to ISO 23753-1:2005. 1 g of soil with natural moisture content was weighted in a test tube, and 1 mL of TTC was added (1 mL of 0.1 M Tris buffer in the control sample). The test tubes were shaken and sealed with a rubbery lid. After 18 h of incubation at room temperature, 5 mL of acetone was added to extract TPF and incubated under dark conditions for 2 h. Test tubes were shaken every hour during the incubation. In the dark, samples were filtered through wrinkled filter paper (slow filtration 90 s – 100 s) absorbance was measured with spectrophotometer Cary 50 Probe UV-VIS (Varian Australia, Mulgrave, Australia), with acetone as a blank. Dehydrogenase activity (*DA*) was calculated using Equation 6.

$$DA = ((\rho_{CS} - \rho_{BS})\ x\ V)/(m\ x\ (SMC\ /100)\ x\ t_{incubation}) \qquad Eq.\ 6$$

DA was expressed as µg TPF per g of solid matter, ρ_{CS} represents the average concentration of TPC in parallel samples in µg mL^{-1}, ρ_{CS} represents the average concentration of TPF in the blank sample, *V* volume of solution in mL, *t* time of incubation, *m* initial mass of sample in g and *SMC* soil moisture content (Section 2.3.2).

2.3.6 Morphological properties

To observe the morphological properties of chitosan-based films, the samples from every stage of deterioration and degradation experiment were photographed with a camera. Afterwards, a small piece (≅ 1 × 0.8 cm) of dried sample was set onto carbon tape, and surface morphology was observed by scanning electron microscope SUPRA 35VP (Carl Zeiss, Jena, Germany).

3 Results and discussion

3.1 Chitosan-based films

In this study, chitosan-based films were prepared as a possible replacement of petroleum-based plastics used for food packaging. Two types of chitosan-based films were made: pure chitosan-based film (CH) and chitosan-based film enhanced by the addition of *Quercus* extract (CHQ) to increase antioxidative and antimicrobial properties. CH films were transparent, while CHQ films had slightly yellow-brownish colour due to incorporated *Quercus* extract (Figure 4). The thickness of CH and CHQ was 40 ± 1 µm and 52 ± 1 µm, respectively. Chitosan-based films demonstrated sufficient mechanical, antioxidant, and optical properties (Figure A.3). In terms of resistance, both types of films are comparable to low-density polyethylene (LDPE) (Mangaraj et al., 2009), which is used to produce plastic wraps. However, chitosan-based films have higher Young's modulus and are less elastic. Both films (CH and CHQ) had similar *MC* of 30 ± 4% and 32 ± 3%, respectively (Table A.1).

Furthermore, both types of chitosan-based films contained polyphenols and exhibited antioxidant properties (Figure 1 and Figure A.2). CH exhibited a lower amount of *TPC* (0.31 ± 0.04 mg_{GAE} g $film^{-1}$) than CHQ (3.6 ± 0.2 mg_{GAE} g $film^{-1}$). Polyphenols bind to chitosan and cause high resistance of such film. Polyphenols might even act as a bridge, binding with more than one chitosan molecule, which causes an increased density (Siripatrawan and Harte, 2010). *TPC* did not change considerably throughout 19 days (Figure 1). The addition of extract improves optical barrier properties (Figure A.3), which is important for the photostability of specific food compounds (Duncan and Chang, 2012). We did not observe any major differences in *MC* in both types of films; however, some studies indicated that the addition of *Quercus* extract could lead to the increase of water content in films due to the possible polyphenols and chitosan interactions through hydrogen bonding. Due to the competitive binding effect, this may result in limited interaction of hydrophilic groups in chitosan with water molecules (Siripatrawan and Harte, 2010; Sun et al., 2017). The solubility of films is of critical importance for its application as a protective barrier in packaging and thus, films should be water-resistant. On the other hand, low solubility is often linked to low biodegradability (Sweetlove et al., 2016). Both chitosan-based films exhibit *TSM*: 23 ± 4% and 24 ± 1% for CH and CHQ, respectively, while conventional petroleum-based plastics such as LDPE does not swell in water and is insoluble (Vasile and Pascu, 2005). Similarly, there was no major difference in films regarding water vapour transmittance and water vapour permeability, thermogravimetric properties and elemental composition (Table A.2 and A.3, Figure A.4).

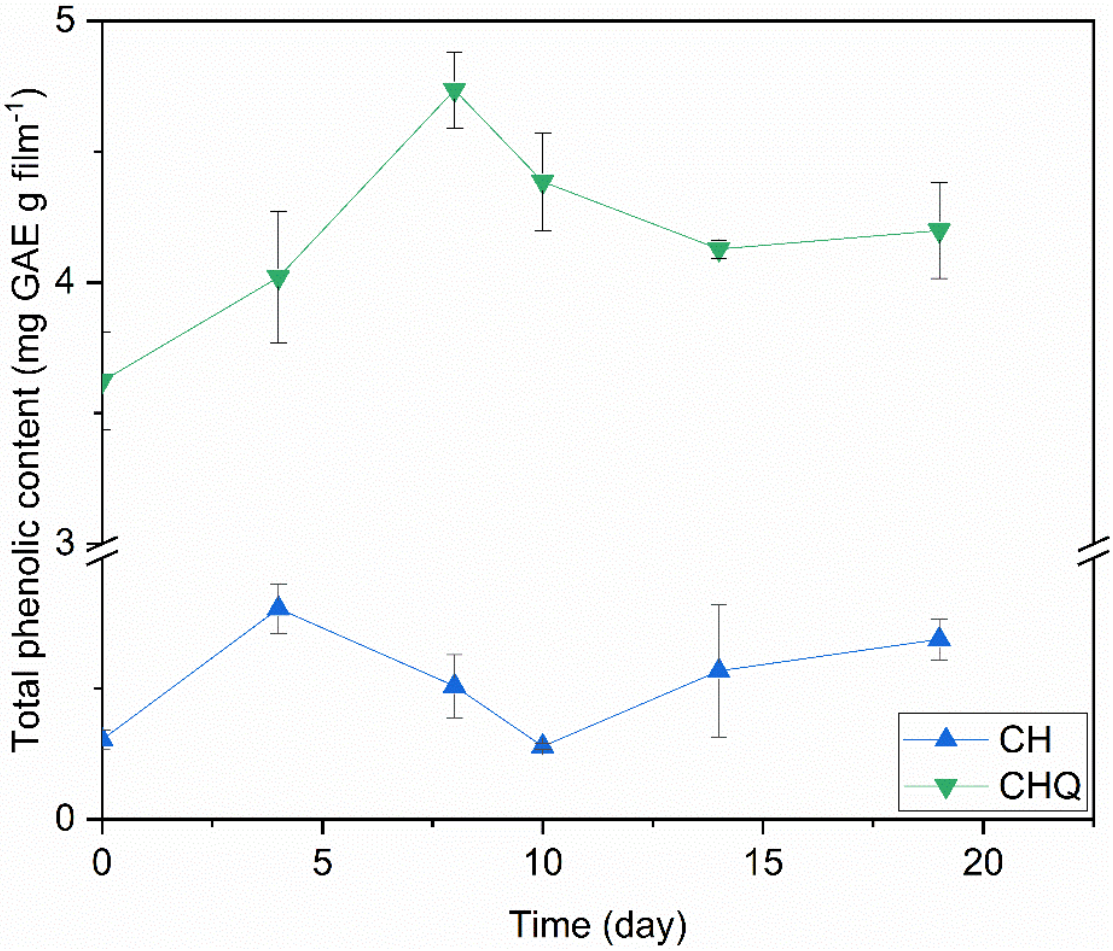

Figure 1: TPC of CH and CHQ over 19 days.

3.2 Biodegradability evaluation

3.2.1 Soil properties

The compositions of industrial compost, commercial garden and vineyard soils are presented in Figure 2. The water content in industrial compost and garden soil was similar (57.9 and 59.3 %, respectively), while vineyard soil contained a significantly lower amount of water, only 25.0%. As expected, the values for the organic matter are 5 to 8 times higher in industrial compost and commercial garden soil in comparison to the vineyard soil, which has the highest percentage of inorganic matter (71.5%) compared to the 26.3% in industrial compost and 16.7% in garden soil, respectively. Further analysis of inorganic matter is presented in Table A4. In all the soils, SiO_2 and $CaCO_3$ are mostly represented with the values of 65%, 85% and

97% for the garden, compost and vineyard soils, respectively. Only in vineyard soil Fe oxides and bornite are being detected, which can explain the colourization of the film during the degradation.

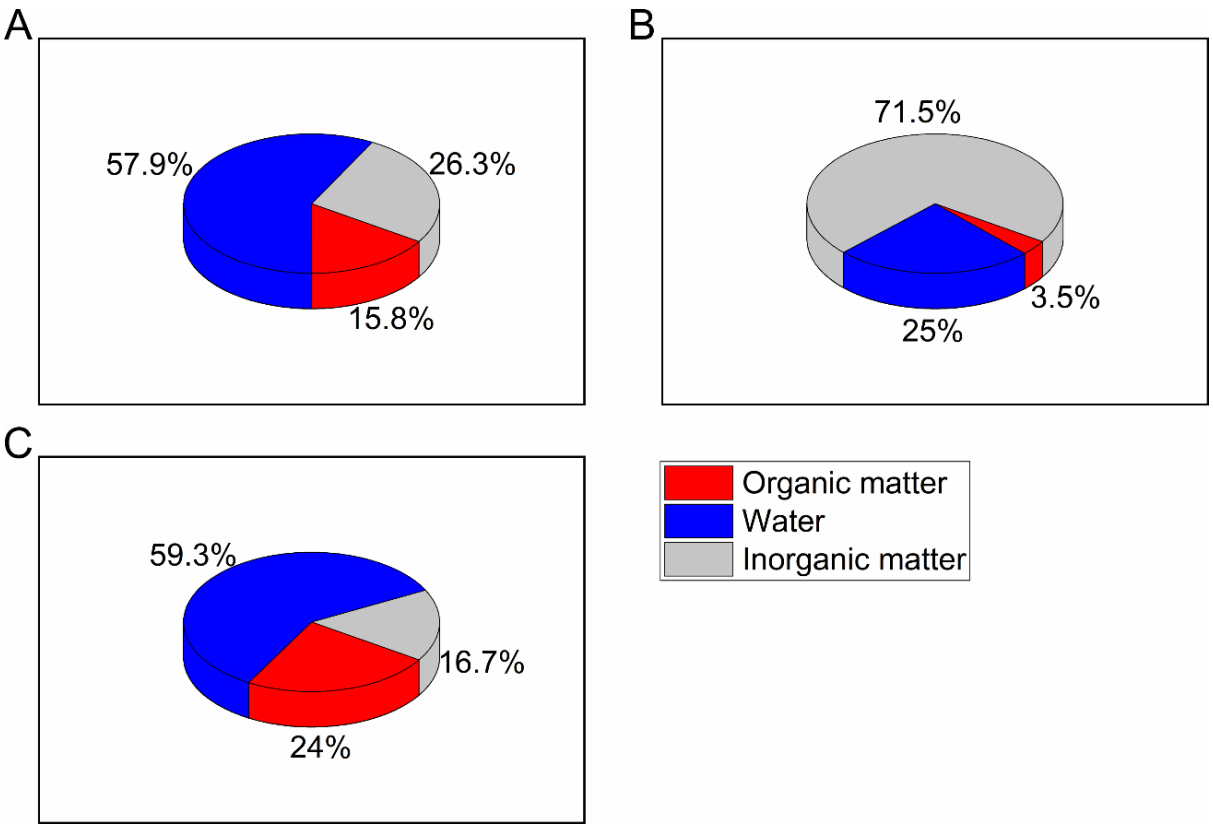

Figure 2: Composition of: A) industrial compost, B) vineyard soil and C) garden soil.

Enzymatic activity is known to be a major indicator of biological activity in soils, considering organic matter metabolism (Bohacz, 2019; Szwed and Bohacz, 2014). As enzymatic activity is strongly dependant on moisture, oxygen content, tillage, the content of organic carbon and nitrogen and presence of heavy metals (Dick, 1992; Krzywy-Gawrońska, 2012; Piotrowska-Długosz and Wilczewski, 2014), values may vary depending to soil type, cultivation, fertilization, the season of measurement, and in case of industrial compost, stating materials (Szwed and Bohacz, 2014).

As can be seen in Figure 3A, urease activity was similar for all types of soil. However, industrial compost exhibited the highest dehydrogenase activity in comparison to other soils (Figure 3B). This could be expected since industrial composts usually contain the most vivid microorganisms and thus a high load of organic matter and optimal conditions for degradation. On the contrary, vineyard soil showed the lowest dehydrogenase activity. The soil was sampled in autumn and was not fertilized before the sampling. The application of pesticides should not cause low activity of dehydrogenase since the majority of them do not affect dehydrogenase activity or their inhibition (Deborah et al., 2013).

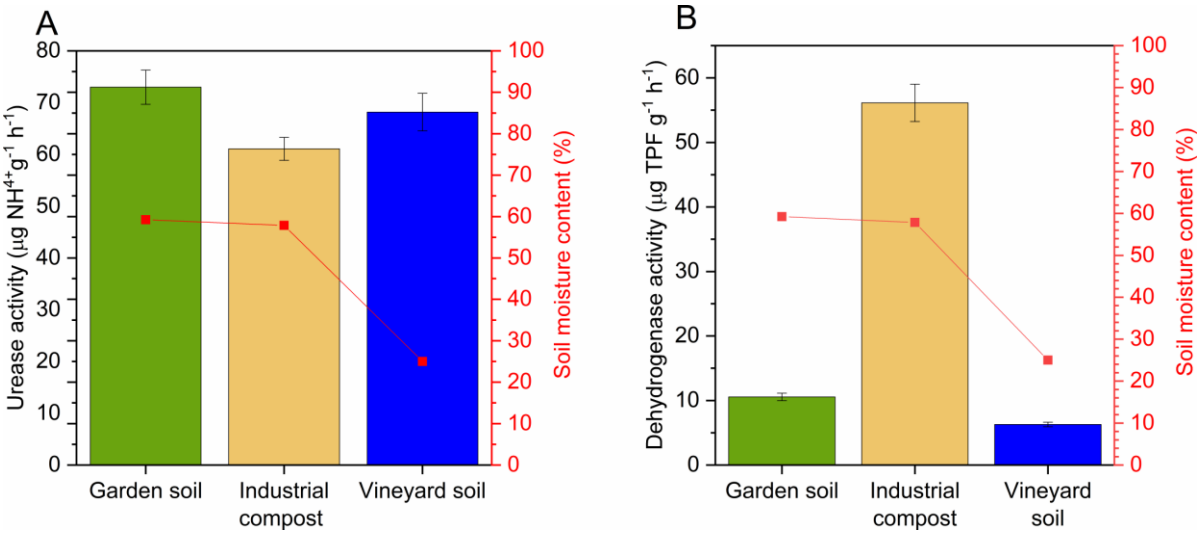

Figure 3: A) Urease activity and B) dehydrogenase activity with soil moisture content.

3.2.2 Morphological properties of chitosan-based films

Degradation of chitosan based-films in soil was firstly observed by the naked eye (Figure 4). Already after three days, both types of chitosan-based films (CHQ and CH) were wrinkled in industrial compost, whereby CH was already fragmented and partially degraded (missing bits at sides). However, in vineyard soil, CHQ did not wrinkle and was practically unchanged, while CH broke down in smaller pieces. CH transformed into a gel-like structure in garden soil after three days, which could be attributed to the disruption of original structure due to degradation and increased swelling, while CHQ kept its original form but reduced in thickness and loss of

colour occurred. Since colour is provided to the film by the *Quercus* extract, it can be concluded that loss of extract in the chitosan matrix occurred. Furthermore, the furrowed surface of films can also indicate the initial degradation of films (Figure 5).

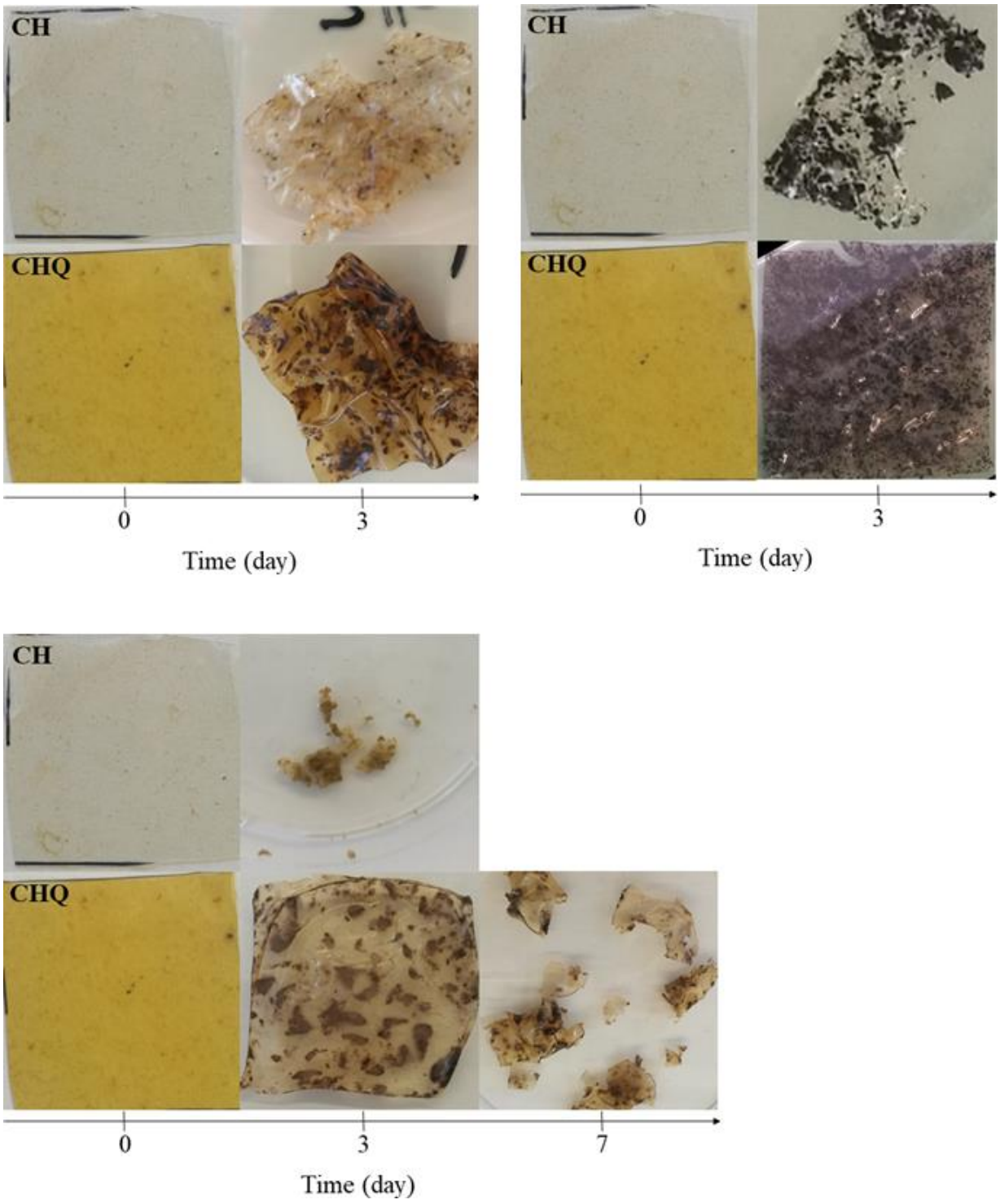

Figure 4: Visualisation of CH and CHQ during three days of biodegradation in industrial compost (upper left), CH and CHQ during three days of biodegradation in garden soil (upper right) and seven days of degradation in vineyard soil (bottom).

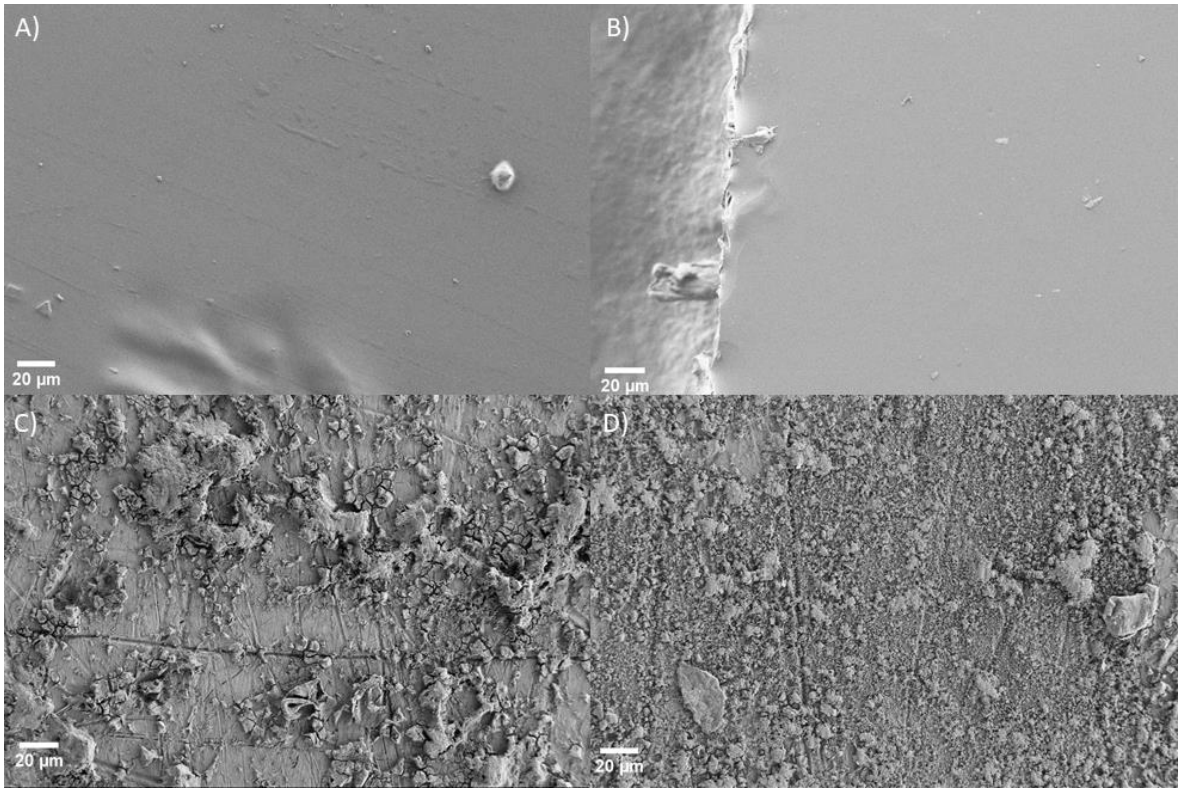

Figure 5: Surface of: A) CH before degradation; B) CHQ before degradation; C) CH after three days of degradation in industrial compost; D) CHQ after three days of degradation in industrial compost.

3.2.3 Degree of biodegradation and total phenolic content

Chitosan-based films prepared in this study were exposed to animate and inanimate activity in three soils under laboratory conditions. The degradation proceeded in soils with natural moisture and with the addition of water to simulate natural precipitation. In the process of biodegradation, first fragmentation occurs. As a result of chemical destruction and the influence of either animate of inanimate factors, the chitosan-based films mechanically disintegrate. The products of this process are then mineralized by microorganisms, where the metabolism of partly fragmented polymers to the end products takes place. It is also possible for some material to degrade into smaller pieces or fragments under UV or thermal influence, but the conversion from organic to inorganic carbon does not occur. Biodegradation of CH

proceeded faster in comparison to CHQ in soils with moisture content (NMC) as well as in soils with added water (AW) as shown in Figure 6. However, the addition of water decelerated the biodegradation in industrial compost and vineyard soil, while in commercial garden soil, the change was not altered. A similar trend was observed with CHQ. Considering that *TPC* impacts antioxidant as well as antimicrobial properties (Bajić et al., 2019a; Kõrge et al., 2020b; Siripatrawan and Harte, 2010), the higher concentration of *TPC* in CHQ is the most probably responsible for the lowering of the rate of biodegradation of tested films.

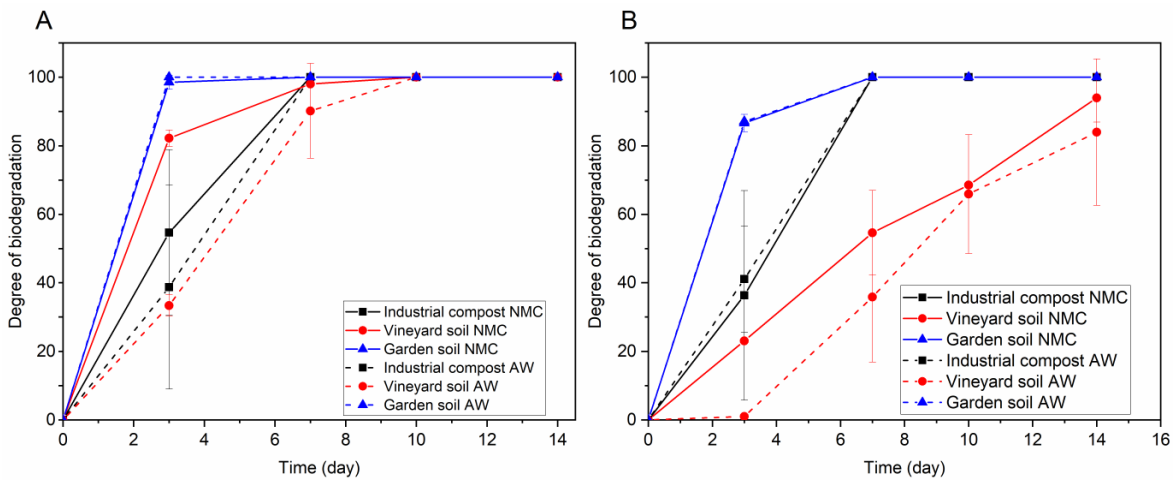

Figure 6: Degree of biodegradation of A) CH and, B) CHQ in NMC and in AW.

In industrial compost and garden soil, the *TPC* in chitosan-based films decreased as degradation proceeded in both tested soil humidity (Figure 7). It was concluded that *Quercus* extract in CHQ degrades well in these two types of soil. However, *TPC* did not decrease in accordance to gravimetrically determined degradation in vineyard soil. Despite the loss of sample mass, TPC decreased from 4.7 mg_{GAE} g $film^{-1}$ to 2.9 mg_{GAE} g $film^{-1}$ in NMC (2.6 mg_{GAE} g $film^{-1}$ in AW), which is roughly 35%. Results are indicating, that in vineyard soil, the CHQ films are degraded at a slower rate than chitosan film itself. We assume that due to low humidity of vineyard soil, the water-soluble *Quercus* extract was unable to dissolve in soil moisture that consequently led to low bioavailability and reduced biodegradation of CHQ (Jordão et al., 2017).

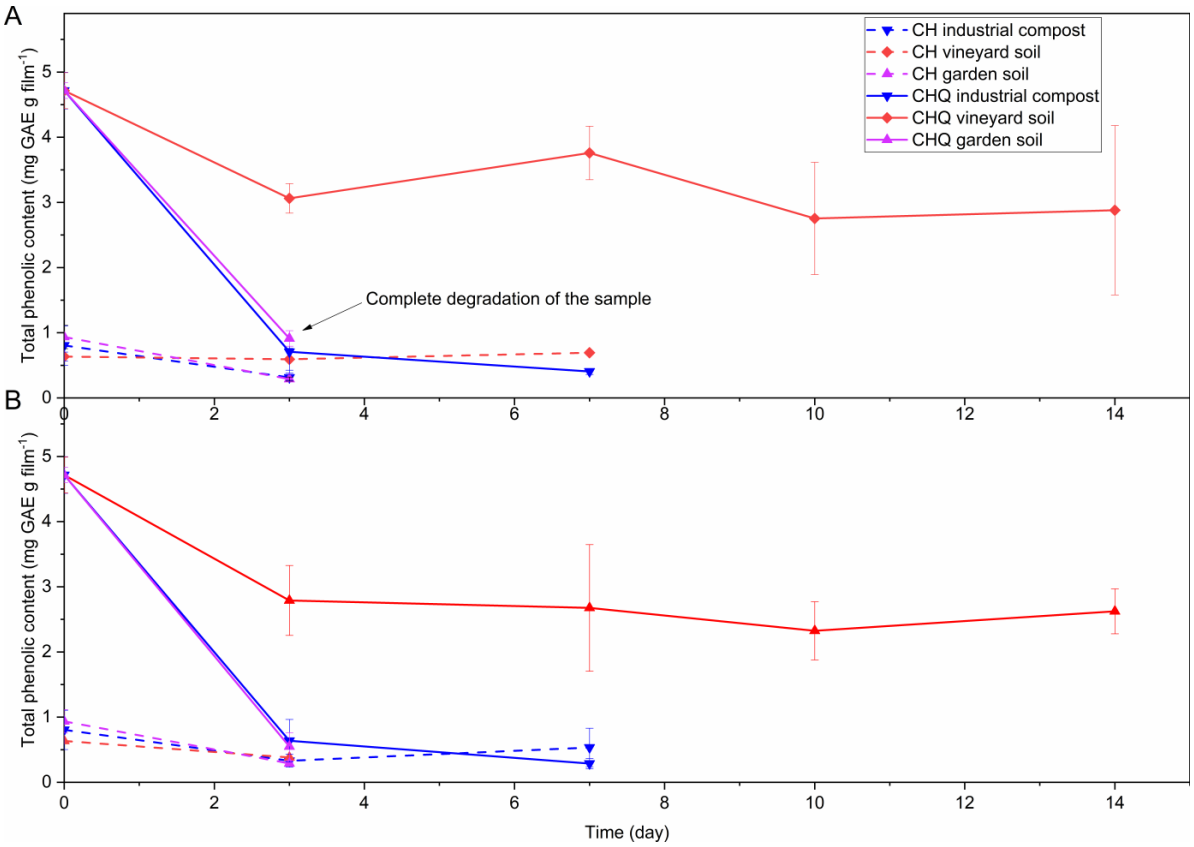

Figure 7: TPC of CH and CHQ in A) soil with natural moisture content and B) soil with added water.

3.2.4 Fourier-transform infrared spectroscopy analysis of degradation

All samples were analysed after degradation, however, there was no major difference observed in spectra between different types of soil. In Figure 8:, the spectra of samples from industrial compost are presented. Band between 3600 cm^{-1} and 3200 cm^{-1} is correlated to stretching O−H and N−H vibrations. The band around 2870 cm^{-1} corresponds to C−H stretching. With degradation, a change is visible in this range. Therefore, a decrease in the amount of C−H bonds can be assumed. A noticeable change was also observed at the band around 1650 cm^{-1}, which correlates to C=O stretching vibrations. Chitosan was not completely deacetylated, this change could be attributed to further deacetylation while degradation (Bagheri-Khoulenjani et al., 2009). A decrease in intensity of bands around 1550 cm^{-1}, 1375 cm^{-1}, and 1025 cm^{-1} (which correlates to N−H, C−N, and C−O stretching, respectively), indicates cleavage of amine,

amide, and carboxylic groups. The decrease in the concentration of C−O bonds can also indicate the decomposition of chitosan onto smaller units (Beier and Bertilsson, 2013). However, due to a decrease in the mass of samples, the entire mechanism of degradation could not be discerned based on FT-IR spectra only.

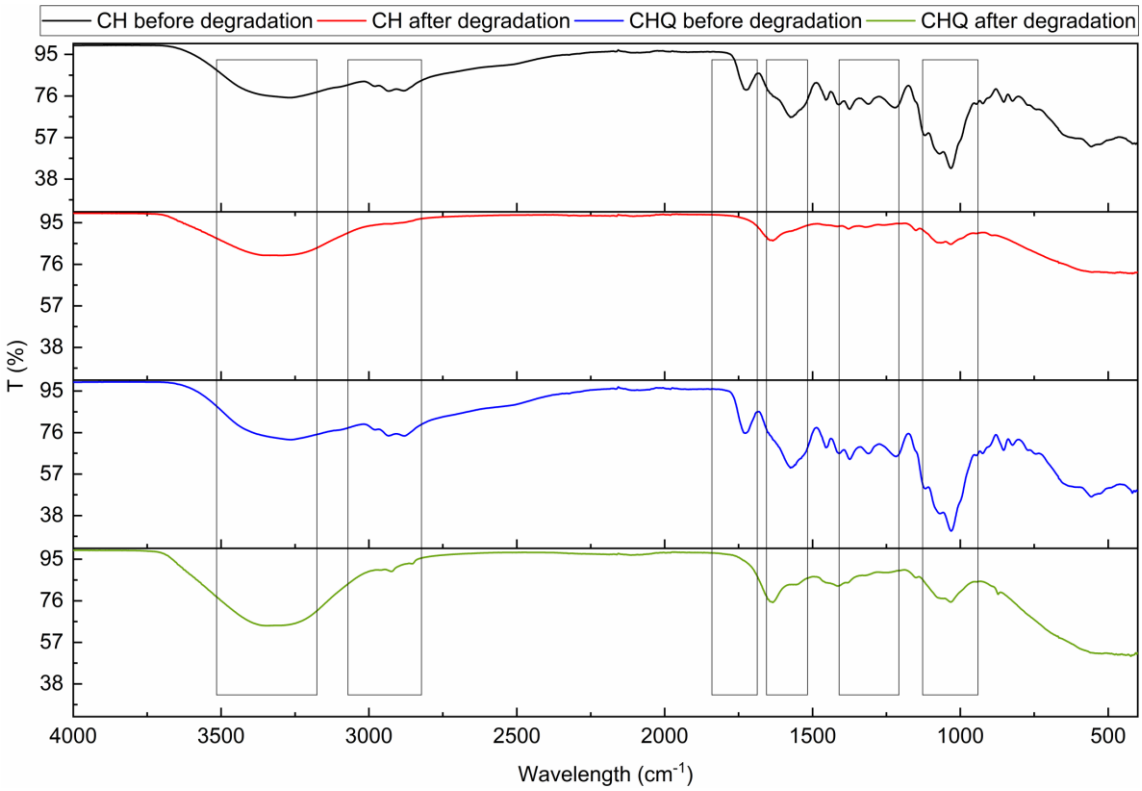

Figure 8: FT-IR spectra of CH and CHQ before and after 3 days of degradation in industrial compost.

4 Conclusions

With the modern world's endless demand for plastic products, the problem of plastic waste has become a severe issue. At the same time, a great number of valuable biopolymers are discarded as industrial waste every day. This study, for the first time, evaluates the degradation of chitosan-based active packaging material in soils.

The material showed complete fragmentation and degradation in industrial compost and gardening soil over the period of 7 days and in vineyard soil over 10 days. It was also observed that addition of water into the soil slowed the process of degradation and therefore in an instance of commercial use of such films as food packaging, further analyses regarding their degradability in different seasons and temperature regimes and aqueous environments as well as after-use degradability of films in higher concentrations should be further investigated. Moreover, the deterioration and fractionation effect on the packaging is seen already after a couple of days regardless of the water content, which can limit the packaging potential to be used for longer periods if there has been a contact with the soil's microorganism.

Nevertheless, in comparison to currently used low-density polyethylene (LDPE) films which are degraded at rate 0.2 wt% per 10 years (A.C. Albertsson, 1980), the key benefits of this packaging are low impact on the terrestrial environment leaving no trace as well as significantly reducing both food processing industry waste and plastic waste.

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