N-hexylimine-chitosan, a biodegradable and covalently stabilized source of volatile, antimicrobial hexanal. Next generation controlled-release system

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Abstract

In this paper, a new type of controlled-release system was prepared by the covalent attachment of a volatile antifungal agent, hexanal, to the biodegradable polymer chitosan. Polysaccharide chitosan was reacted with hexyl aldehyde in Schiff base reaction to form iminated N-hexylimine-chitosan (NHIC). Physical and mechanical properties of the modified polymer films were studied and compared to those of unmodified chitosan. An aqueous solution of HCl (3M) was used to catalyze the hydrolysis of imine bonds, to yield regenerated chitosan and released hexanal. The release of hexanal was examined using headspace gas chromatography. The antifungal activity of N-hexylimine-chitosan film was demonstrated on harvested wheat. Acidic stimulation resulted in release of hexanal in the grain storage container and a significant (up to 10-fold) decrease in the mold occurrence on the grain.

The presented controlled-release system is based on dynamic covalent bonding. The formation of a stable covalent bond completely prevents the escape of active compound and can, however, be easily hydrolyzed. This approach may be a considerable tactic for the controlled release of volatile active agents.

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1. Introduction

In the past decades, significant progresses have been made in the development of active biodegradable polymeric materials (Nair & Laurencin, 2007). These polymers are promising candidates for use in tissue engineering, packaging materials, bioactive delivery vehicles, and controlled/sustained-release systems. The development of biodegradable systems capable of controlled-release of active agents is of great scientific interest and has practical applications for drug delivery, the flavor/aromatization industry, and the cosmetic industry (Cha & Chinnan, 2004; Desai & Park, 2005). Biodegradable polymeric materials that can be used for the controlled release of active agents are also desired in the food industry. Such controlled-release systems may enhance the quality of food products and prolong their shelf-lives (Lopez-Rubio, Gavara, & Lagaron, 2006). For instance, fungi and concomitant mycotoxins remain a serious issue during storage and processing of wheat-derived products. Volatile antimicrobial agents such as low molecular weight fatty acids (Cagri, Ustunol, & Ryser, 2004; Mani-Lopez, Garcia, & Lopez-Malo, 2012), aldehydes (Nandi, 1977; Power, 1997), and essential oils (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009; Espitia, Du, Avena-Bustillos, Soares, & McHugh, 2014) have been shown to significantly reduce the development of harmful fungi in grain. Among these is hexanal, an aldehyde product of lipid oxidation and peroxidation in butter, human milk, and vegetable oils (Elisia & Kitts, 2011; Panseri, Soncin, Chiesa, & Bianchi, 2011). Hexanal has been approved for use as a food additive by the FDA [EAFUS: A Food Additive Database Center of Food Safety and Applied Nutrition, US Food and Drug Administration (FDA), 2006] and has been shown to have antimicrobial activity (Andersen et al., 1994; Lanciotti, Corbo, Gardini, Sinigaglia, & Guerzoni, 1999; Musetti & Fava, 2012; Nandi, 1977; Song et al., 2007). However, the use of highly volatile substances such as hexanal is quite problematic and limited. The activity of such compounds is fairly transitory, which requires the continuous addition of these active supplements. In addition, direct addition of pure active compounds may cause deterioration of the food product or corrosion of the container in which it is kept.
Therefore, there is great interest in systems for the controlled release of such volatile active agents.

Various controlled-release systems based on biodegradable polymers have been described (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010). These systems utilize reversible non-covalent interactions to encapsulate and then release the active agent. Encapsulation of active agents is promoted by hydrogen bonding and hydrophobic or electrostatic interactions. The release is usually triggered by a change in pH, temperature, or humidity (Almenar, Auras, Rubio, & Harte, 2007; Saenz-Garza, Delaquis, & Durance, 2013). In the case of volatile active components, controlled-release systems based on non-covalent interactions inhibit the release rate and help to prolong the activity of the encapsulated agents, but cannot completely prevent their evaporation. Such systems should be stored in hermetically sealed flasks under appropriate humidity and temperature conditions (Saenz-Garza et al., 2013). Dynamic covalent bonds are bonds that can break and reform under appropriate conditions without side reactions (Guan & Zhang, 2014; Sanchez-Sanchez & Pomposo, 2014). In this research, we aimed to generate a new controlled-release system, in which an active compound is covalently attached to a biodegradable substrate. The approach may avoid undesired spontaneous release and allow precise control of an amount of the released active agent. In addition to a precise controlled release, dynamic covalent binding allows safe delivery of volatile agents. Volatile antimicrobial agents are usually vigorous, have strong odor and present in liquid form. Covalent attachment to a polymer platform makes volatile compounds steady, odorless and allows them to be in solid state. Such approach provides a safe way for delivery and transportation of these active agents. Chitosan, a biodegradable polysaccharide derived from the natural polymer chitin, served as the polymer substrate (Elsabee & Abdou, 2013). Chitosan exhibits good film-forming ability, as well as other physical properties that make it appropriate for this work, such as particular levels of viscosity, solubility in various media and mucoadhesivity (Balamurugan, 2012; Simkovic, 2013). In addition, chitosan also has notable biological properties, including antimicrobial activity. Chitosan has one –NH₂ group and two –OH groups on each of its glucosidic residues. The primary amino group of chitosan provides a specific platform for side-group attachment (Beneditkstottir et al., 2011; Oladoja, Adelagun, Ahmad, Unuabonah, & Bello, 2014; Pinto et al., 2012).

In this work, we utilized amine groups on the chitosan backbone for Schiff base condensation reaction with hexyl aldehyde to form iminated N-hexylimine-chitosan (NHIC). The physical and mechanical properties of the modified polysaccharide were studied and compared to those of unmodified chitosan. Hydrolysis of the imine bond resulted in the regeneration of chitosan and the release of antifungal hexanal, whose activity was confirmed by the inhibition of fungal growth on harvested wheat.

2. Materials and methods

2.1. Materials

Chitosan of medium molecular weight (Poly-β-glucosamine, degree of deacetylation > 85%), hexanal (≥98%), acetic acid (≥98.8%), hydrochloric acid (32–35%), water (HPLC grade), hexane (HPLC grade) were purchased from Sigma Aldrich, Israel. Potato dextrose agar (PDA) was purchased from Difco™ (Becton Dickinson and Co, Maryland, USA).

2.2. Preparation of N-hexylimine-chitosan films

Chitosan (1.5 g, 8.85 mmol) was dissolved in 100 mL aqueous solution of 0.6% acetic acid. Hexanal (3.065 mL, 21.1 mmol) was then added under intensive stirring. The reaction mixture was stirred at room temperature for 24 h. The resulting viscous suspension (5 mL) was poured into Petri dishes (5 mm in diameter) and air-dried at room temperature until good quality films were formed (1.82 g, 7.61 mmol, 85.98% degree of substitution). The N-hexylimine-chitosan films were repeatedly washed with hexane to remove a non-covalently bound hexanal and dried under vacuum. Pure chitosan film was prepared by dissolution of chitosan (1.5 g, 8.81 mmol) in 100 mL aqueous solution of 0.6% acetic acid. The resulting viscous suspension (5 mL) was poured into Petri dishes (5 mm in diameter) and air-dried at room temperature until good quality films were formed.

2.3. Release of hexanal from N-hexylimine-chitosan films by hydrolysis

Aqueous solution of hydrochloric acid (3M, 5 mL) was added to a vial that contained 50 mg of N-hexylimine-chitosan. The vial was sealed and reaction mixture was agitated for 24 h at room temperature. Hexanal release was monitored by GC.

2.4. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra were recorded by the infrared spectrometer (Bruker Tensor 27 FTIR Spectrometer). Each spectrum resulted from 100 scans in the wave number range 400–4000 cm⁻¹. Signal averages were obtained at a resolution of 4 cm⁻¹. The obtained films were directly analyzed after drying in desiccator with no KBr.

2.5. Thermal analyses

Thermal stability and degradation of films were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), performed with a TGA/DSC1 analyzer (Mettler-Toledo, OH, USA). Dry films samples stored in desiccator (19.7% of humidity at room temperature) of 6–10 mg were placed in the balance system. Thermograms were recorded in nitrogen at a heating rate of 15 °C/min over the temperature range 25–600 °C. Weight change and heat flow were measured simultaneously during the analysis.

2.6. Gas chromatography

Hexanal concentration was determined by gas chromatography based on a reported method on a Shimadzu GC-2010 Plus instrument (Kyoto, Japan), equipped with a fused-silica capillary column (Agilent, Netherland). Each sample was incubated and agitated during 3 min at 40 °C before the injection. The injector and detector (FID) temperatures were maintained at 230 and 240 °C, respectively. Measurement time was 15 min. Temperature of oven rose from 40 to 240 °C, with initial hold time of 5 min at 40 °C. Samples (1 ml) were injected with 1:5 split ratio, the flow rate of helium as carrier gas was 16.9 mL/min. The reported values were quantified according to a hexanal calibration curve.

Into a 25 mL vial with 50 mg of N-hexylimine-chitosan was added 5 mL of 3M HCl as described previously. The vial was sealed with a septum cup. To detect release of hexanal during 24 h with 1 h interval, 25 identical vials were prepared and stored at identical conditions. Every hour 1 mL gas sample was taken from the vial head space, and injected to a GC. A control experiment was done. N-hexylimine-chitosan films (50 mg) were dipped into 5 mL of water and in parallel to the previous experiment 25 vials were sampled every hour, during 24 h.
2.7. Film thickness

Film thickness (μm) was determined on the films averaging measurements at five points for each film using a hand-held microcaliper (Mitutoyo Corp, Kawasaki, Kanagawa, Japan).

2.8. Moisture content

Three specimens of each film were weighed (m_w) and subsequently dried in an air-circulating oven at 105 °C for 24 h. Films were then reweighed (m_0), to determine their moisture content (MC):

\[
MC(\%) = \left( \frac{m_w - m_0}{m_w} \right) \times 100
\]

2.9. Tensile strength (TS), elongation at break (EB) and Young’s modulus

TS, EB and Young’s Modulus were determined using an LR-5K tensile testing instrument (LLOYD, UK). The TS was expressed in MPa and was calculated by dividing the maximum load (N) by the cross-sectional area (m^2). EB was determined by dividing the extension at the moment of rupture by the initial gauge length of the samples and multiplying by 100. Young’s modulus was determined by ratio of the stress along an axis over the strain along that axis in the range of stress. TS, EB and Young’s modulus measurements were replicated ten times for each type of film.

2.10. Binocular microscope

Fungus development was observed through binocular microscope (model SDZ-P, Kyowa Optical Co., LTD, Tokyo).

2.11. Atomic forth microscopy (AFM)

Topographic imaging was performed using Innova AFM with a NanoDrive Controller (Bruker, California) operating in the tapping mode, in air, at room temperature. Surface images, using scan widths ranging from 1 μm to 5 μm, with a scan rate of 1.0 Hz were acquired at fixed resolution (512 × 512 data points). Bruker 0.01–0.025 Ohm-cm Antimon (n) doped silicon tips (model: RTESPA-CP) were used. The roughness parameter such as the root mean square (R_q) was calculated for scanned area (5 × 5 μm) using NanoScope Analysis software. The AFM images and roughness calculations were obtained for different sample places and the most typical areas are presented.

2.12. Antifungal activity of N-hexylimine-chitosan during wheat storage

To a sterile 250 mL beaker 50 wheat grains were added. Different types of treatments were examined. To the beaker with grains were added (a) 200 mg of NHIC films, (b) 200 mg of NHIC films in 5 mL of 3M HCL, (c) 150 μL of pure hexanal, (d) 5 mL of 3M HCL, (e) control with no treatment additives. None of the treatments was in direct contact with the grains. The treatment additive was placed in separate open vial inside the beaker. All types of treatments were tested in triplicate.

Each beaker was sealed and placed in incubator at 29 °C for 30 days. After that the beakers were unsealed and the grains from each beaker were placed on PDA plates (prepared with 0.005% of chloramphenicol), 10 grains on each PDA plate. Fungi mold growth on each grain was checked every 24 h under binocular microscope and the percent of infected grains on each plate was found.

3. Results and discussion

3.1. Formation and characterization of N-hexylimine-chitosan (NHIC)

The generation of an NHIC derivative of chitosan requires a one-step synthesis in which the aldehyde functional group of hexanal reacts with the amino group of chitosan via a Schiff base reaction (Scheme 1). The synthesis of alkylated chitosan via reductive amination has been widely discussed in the literature. This method was suggested earlier by Yalpani and Hall (1984) and modified by Rinaudo and colleagues (Rinaudo, Auzely, Vallin, & Mullagaliev, 2005). It involves the reaction of an aldehyde with chitosan in acidic solution to form the chitosan-iminium ion, followed by reduction with sodium cyanoborohydride to yield the secondary amine and alkylated chitosan (Ortona, D’Errico, Mangiapia, & Ciccarelli, 2008; Rinaudo et al., 2005). In this work, we were interested in stopping the process at the imine stage, to allow the regeneration of chitosan and the release of hexanal. Hexanal is released as a result of the acidic hydrolysis of an imine bond (Scheme 1).

The covalent attachment of hexanal to chitosan allows precise control of the release. As mentioned above, a release system for hexanal (or other volatile molecules) that is based on non-covalent interactions needs to be protected (sealed) until the moment of usage and usually cannot completely prevent the escape of active compound. The presented approach is based on the formation of a stable covalent bond, which can, however, be easily hydrolyzed. Such dynamic covalent bonding may be in many cases the most appropriate tactic for the controlled release of volatile active agents.

FTIR spectra of chitosan (prepared by addition of hydrochloric acid), recovered chitosan (from solution of hydrochloric acid), chitosan (prepared by addition of acetic acid) and NHIC (prepared by addition of acetic acid) films are shown in Fig. 1 (A–D). The characteristic absorption bands of pure chitosan (Fig. 1) A and C are as follows (De et al., 2006; Wan, Wu, Yu, & Wen, 2006): (a) broad band at 3500–3100 cm⁻¹ attributed to N–H and OH stretching vibrations overlaying each other, as well as intermolecular hydrogen bonding of chitosan molecules, (b) weak bands at 2879 and 2924 cm⁻¹ from the C–H stretch of CH₂ and CH₃ groups, (c) a peak at 1650 cm⁻¹ that is attributed to the stretching vibrations of amide group carbonyl bonds C=O and (d) the amine-NH₂ absorption band at 1525 cm⁻¹ for films prepared with addition of hydrochloric acid and 1561 cm⁻¹ for films prepared by addition of acetic acid. The FTIR spectrum of NHIC (Fig. 1) D has two strong, sharp peaks at 2924 and 2879 cm⁻¹. These peaks point to an increase in C–H groups due to the addition of C₆ alkyl chains from the bound hexanal. An additional peak with increased intensity at 1657 cm⁻¹ slightly overlaps an amide band at 1650 cm⁻¹ that is attributed to C=NH stretching (imine). This result indicates that the Schiff base reaction with hexanal involved the NH₂ groups in the chitosan. It is worth noting that no peaks related to the aldehyde group of the free hexanal (around 1720–1740 cm⁻¹) were detected. This observation confirms that there was no spontaneous release of hexanal from the NHIC. As expected, the FTIR spectrum of recovered chitosan (Fig. 1) B is similar to the spectrum of the chitosan prepared by addition of hydrochloric acid (Fig. 1) A.

3.2. Physical and mechanical properties of N-hexylimine-chitosan films

The effects of covalent linkage of hexanal on the mechanical and physical properties of the modified chitosan were examined.
3.2.1. Mechanical properties

The thickness, tensile strength (TS), elongation at break (EB), and Young's modulus values of the modified and original chitosan films are shown in Table 1. Young modulus specifies the stiffness or rigidity of the film,TS indicates the tensile strength of the film (i.e., how well it resists breaking) and EB describes the flexibility or extensibility of the films.

Film thickness of chitosan and NHIC films were 46.8 and 67.6 μm for NHIC, respectively.

The attachment of hexanal caused only a slight decrease in the tensile strength (TS) of the polymer film (67.1 MPa for chitosan vs. 55.5 MPa for NHIC). For comparison, the TS values of widely used plastic films, LDPE and HDPE are 23.6 and 47.4 MPa, respectively (Standard method for testing tensile properties of thin plastic sheeting; Philadelphia, PA 2002). The extensibility of the films was barely affected by the attachment of hexanal. The EB of chitosan was 3.3% and the EB of NHIC was 2.9%. Compared to the EB values of LDPE and HDPE (205% and 570%, respectively), both chitosan and NHIC films exhibited poor elongation. Finally, the attachment of hexanal had no dramatic effect on the Young's modulus of the film (2837 MPa for NHIC vs. 3527 MPa for chitosan). Thus, the presented chitosan modification caused only slight alterations in the mechanical properties of the polymer. This observation is of great significance. Mechanical properties of films are largely associated with the distribution and density of intermolecular and intramolecular interactions in the polymer network. The mechanical and physical properties of polymers could be injured upon chemical modification, since such reactions cause structural changes (Young & Lovell, 2011). For example, Barber, Kelley, Griggs, Wallace, and Rogers (2014) reported that chemical modification of chitosan with 4-chlorobutynitrile had a slight negative effect on its physical properties.

3.2.2. Physical properties

At the macroscopic scale, the chitosan and NHIC films were homogenous, robust, and quite flexible. The thermogravimetric analysis of the modified NHIC film and the unmodified chitosan film is illustrated in Fig. 2. The TGA thermogram of the chitosan [Fig. 2(a), solid line] shows two main decreases in weight: a moderate one (ca. 8% weight loss) between 30 and 125 °C and a steep one (ca. 58% weight loss) between 250 and 600 °C, indicating a two-step degradation process. The total weight loss of the sample at about 600 °C was 66%. The DSC thermogram of the chitosan film [Fig. 2(b), solid line] revealed a broad endothermic peak centered at

![Scheme 1. Synthesis and hydrolysis of N-hexylimine-chitosan.](image)

![Fig. 1. FTIR spectra of (A) chitosan (with HCl), (B) recovered chitosan (with HCl), (C) chitosan (with acetic acid) and (D) N-hexylimine-chitosan (with acetic acid).](image)

![Table 1](image)

<table>
<thead>
<tr>
<th>Film type</th>
<th>Thickness [μm]</th>
<th>TS [MPa]</th>
<th>Young's modulus [MPa]</th>
<th>EB [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>46.8 ± 6.7</td>
<td>67.1 ± 6.5</td>
<td>3527.2 ± 311.0</td>
<td>33 ± 1.3</td>
</tr>
<tr>
<td>NHIC</td>
<td>67.6 ± 6.3</td>
<td>55.5 ± 5.5</td>
<td>2836.8 ± 211.1</td>
<td>2.9 ± 0.4</td>
</tr>
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![Fig. 2. TGA (A) and DSC (B) thermograms of the NHIC film (dashed line) and the chitosan film (solid line).](image)
about 70 °C. This peak is attributed to the loss of water associated with the hydrophilic groups of the polymer (Cheung, Wan, & Yu, 2002; Gonzalez, Guerrero, & Ortiz, 2000). This observation suggests that the sample was not completely anhydrous, which was confirmed by the results obtained by TGA analysis. The exothermic peak, which appears between about 280 and 340 °C, corresponds to the decomposition of the polymer (Sakurai, Maegawa, & Takahashi, 2000; Zeng, Fang, & Xu, 2004), matching the weight-loss processes presented in TGA.

The TGA thermogram of the NHIC film [Fig. 2(a), dashed line] shows two main decreases in weight: the first between 30 and 125 °C (ca. 13%), followed by an additional moderate loss between 125 and 600 °C (ca. 53%). The total weight loss of the sample at about 600 °C was 66%. The DSC thermogram of the NHIC film [Fig. 2(b), dashed line] revealed a broad endothermic peak centered at about 85 °C. Similarly to chitosan, this peak is attributed to the evaporation of water. The exothermic peak corresponding to the decomposition of the polymer appeared between about 270 and 330 °C. According to both TGA and DSC, the decomposition of the obtained polysaccharide occurred at a temperature similar to the temperature at which chitosan decomposed.

In order to confirm that the initial weight loss was due to the evaporation of water from the films, a moisture content (MC) experiment was performed (see materials and methods section). The MC value for chitosan was 9.82 ± 0.48% and the MC value for NHIC was 15.34 ± 0.53% for NHIC. The MC values for both films are similar to the initial weight losses measured by TGA.

The results indicate that the thermal stability of NHIC was only slightly affected by the introduction of the hexylimine moiety into

Fig. 3. AFM micrographs (scale bar = 5 × 5 μm) of chitosan (A) 2D and (B) 3D presentations with a depth scale from 1.1 to 11.2 nm; and NHIC in (C) 2D and (D) 3D presentations with a depth scale from −22.3 to 106.9 nm.

Fig. 4. Release of hexanal as detected by headspace GC.
the chitosan chain, as compared to that of the unmodified chitosan film.

3.3. Morphological studies

The morphology of the examined films is shown in Fig. 3. The AFM images show differences in the surface properties of the chitosan film and its modified derivative, NHIC. The surface of the pure chitosan film was considerably smoother than that of the NHIC film. The measured roughness parameter, $R_q$, of the pure chitosan was 2.3. The smoothness of this chitosan film is similar to that reported by Young, Seong, Sang, Heung, and Chun-Ho (2012). Such a flat, smooth surface is a consequence of chitosan crystallinity (Bierzbrauer, Alasino, Munoz, Beltramo, & Strumia, 2014; Mohanasrinivasan et al., 2014). In the case of NHIC, visible changes in the roughness of the surface were noted. The roughness parameter, $R_q$, of NHIC was 31.9. This high $R_q$ indicates an increase in heterogeneity as a consequence of the loss of crystallinity in NHIC. The modification of the chitosan affected the arrangement of the molecules and caused phase segregation or the reorganization of the hydrophobic chains of hexanal to the surface.

3.4. Release of hexanal from the NHIC film

When an aqueous solution of HCl was added to the NHIC film, the imine bond was hydrolyzed to yield free hexanal and regenerated chitosan (Scheme 1). The regenerated chitosan was isolated and characterized by FTIR spectra that included peaks analogous to those of the original chitosan (Kumirská et al., 2010). No peaks were found in aliphatic or double-bond areas, confirming the cleavage of the imine bonds and hexanal release.

Hexanal release was monitored by GC (Fig. 4). HCl solution was added to each of the 25 sample vials containing equal amounts of NHIC film at the same time. The hexanal concentration in the headspace of each vial was measured hourly. The regeneration reaction happened very quickly; after 2 h, there was no significant increase in the concentration of the released hexanal (Fig. 4). The average concentration of released hexanal was found to be $5.16 \times 10^{-5}$ mol/mL gas (50 mg of NHIC release 6.3 $\mu$m of hexanal).

3.5. Inhibition of fungal growth on wheat by hexanal released from the NHIC film

The antifungal activity of the NHIC films was revealed on harvested wheat. Grains can be contaminated by a variety of fungi during postharvest storage (Audenaert et al., 2012). Postharvest disease control has become more challenging due to the limited number of allowed fungicides, fungicide resistance and consumer demand for reduced fungicide residues in food products. Fig. 5 shows the effects of various treatments on the incidence of infected grain during 4 weeks of storage. The following treatments were applied to grain in storage containers: a) NHIC film activated with HCl; b) untreated NHIC film, as a negative control; c) pure hexanal, as a positive control; and d) 3M HCl, in order to verify that the antimicrobial effect of HCl-treated NHIC is due to the released hexanal and not the HCl. A container of untreated grain served as an additional control.

In the case of the absence of any treatment and in the case of the untreated NHIC film, grain spoilage began immediately and reached 43% and 28% after 1 day and 100% spoilage after 2 days. As expected, NHIC films do not release hexanal, due to its covalent bonding. For the grains stored with HCl solution, which is known to have slight fungicidal activity (Basaran, 2011), almost 50% spoilage was observed after 2 days, more than 80% after 3 days, and complete (100%) spoilage was observed after 9 days. Both pure hexanal and the activated NHIC significantly inhibited grain spoilage. A much slower rate of spoilage was detected in the grain treated with pure hexanal, as expected, and in the treatment involving the hexanal released from the NHIC films. Only after 4 weeks of storage, 80% spoilage was observed in the grain treated with pure hexanal and in the grain treated with the activated NHIC. These results indicate that hexanal (pure or released form the NHIC film) has excellent antifungal activity. The activity of hexanal against fungi isolated from wheat grain was described by Nandi (1977).

4. Conclusions

In this paper, we have described a new controlled-release system that was prepared by the covalent attachment of volatile bioactive molecule, hexanal, to the biodegradable polymer chitosan. The modified N-hexylimine-chitosan (NHIC) film was studied and the reported modifications were found to have no dramatic effects on the mechanical and physical properties of the original polymer. Hydrolysis with HCl solution at room temperature caused the rapid cleavage of the imine bond, to yield regenerated chitosan and free hexanal. The activated NHIC film demonstrated significant antifungal activity and significantly inhibited the postharvest spoilage of wheat.

The dynamic covalent linkage of the active agent may provide a new approach for the formation of effective controlled-release system. The clear antifungal action observed in the activated NHIC film may open the way for the development of new coating systems for postharvest protection.
systems. In the present case, we have demonstrated that the modified chitosan film can serve as a biodegradable platform for the controlled release of volatile active agents.

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