

Thermal and antimicrobial properties of chitosan–nanocellulose films for extending shelf life of ground meat



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ABSTRACT

Chitosan–nanocellulose biocomposites were prepared from chitosan having molecular weight of 600–800 kDa, nanocellulose with 20–50 nm diameters and various levels of 30, 60 and 90% (v/w_{CHT}) for glycerol. Agitation and sonication were used to facilitate even dispersion of particles in the polymer matrix. The nanocomposites were examined by differential scanning calorimetry, X-ray diffraction and agar disc diffusion tests; finally, the film was applied on the surface of ground meat to evaluate its performance in real terms. Chitosan–nanocellulose nanocomposites showed high T_g range of 115–124 °C and were able to keep their solid state until the temperature (T_m) range of 97–99 °C. XRD photographs revealed that nanocellulose peak completely disappeared after their addition to chitosan context. Agar disc diffusion method proved that the nanocomposite had inhibitory effects against both gram-positive (*S. aureus*) and gram-negative (*E. coli* and *S. enteritidis*) bacteria through its contact area. Application of chitosan–nanocellulose nanocomposite on the ground meat decreased lactic acid bacteria population compared with nylon packaged samples up to 1.3 and 3.1 logarithmic cycles at 3 and 25 °C after 6 days of storage, respectively.

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1. Introduction

An area of growing interest, these days, is the preparation of antimicrobial edible films applied for controlling foodborne microbial outbreaks mainly caused by minimally processed fresh products (Güçbilmez, Yemenicioğlu, & Arslanoğlu, 2007). Chitosan (CHT), the cationic (1–4)-2-amino-2-deoxy- β -D-glucan, is industrially produced in various quality grades from chitin, the second most abundant polysaccharide in nature (Muzzarelli, 2012; Tome et al., 2013; Muzzarelli et al., 2012). Chitin and chitosan are natural antimicrobial compounds against an extensive variety of microorganisms including bacteria, yeasts and moulds (Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011). Chitosan is a non-toxic and biodegradable compound and has excellent performance in forming films (Mayachiew, Devahastin, Mackey, & Niranjana, 2010). Chitosan films have successfully been used as packaging materials for the preservation of food quality (Fernandes et al., 2009),

which motivated us to select this carbohydrate polymer as a matrix context for preparing desirable edible films.

Cellulose consists of D-glucopyranose units joined together by β -(1→4)-glycosidic bonds and could be found in wood, cotton, hemp, etc. (Khan et al., 2012). Cellulose nanocrystals have low densities, high elongation moduli and tensile strengths (Dadashi, 2011; Klemm, Heublein, & Fink, 2005); besides, they have high biodegradability rates and are less expensive than other nanofillers (Hansson et al., 2013). Nanocellulose (n-cellulose) particles are very suitable nanomaterials for the production of cheap, lightweight, and very strong nanocomposites; meanwhile, they are more effective than their micro-sized counterparts to reinforce polymers (Azeredo et al., 2010).

Nanocomposites (NCPs) are novel polymer matrices which have been incorporated by nanoparticles having at least one dimension in nanoscale (Petersson & Oksman, 2006). At the same time, chitosan–cellulose compounds are of particular interest because of the structural similarity between these two biopolymers (Fernandes et al., 2009). Khan et al. (2012) incorporated 1–10% (w/w) n-cellulose particles with 5–10 nm width into chitosan and analyzed mechanical and barrier properties of prepared

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NCPs. De Mesquita, Donnici, Teixeira, & Pereira (2012) incorporated n-cellulose particles having 145 nm length and 6 nm diameter into chitosan matrix and studied structural characteristics of obtained films. Fernandes et al. (2010) prepared chitosan–nanocellulose NCPs from low and high molecular weight chitosan powders (and their modified ones) and 5–60% (w/w) nanocellulose; they investigated mechanical and thermal properties of prepared nanocomposites and reported that the addition of NCL increased degradation temperatures of chitosan films up to 45 °C. However, there are not any researchers dealing with thermal (compared with synthetic polymers) and antimicrobial properties of this NCP and this has led to some confusion about practical applications of the nanocomposites for industrial uses, having been evaluated in the current research.

Microbial growth commonly imposes undesirable organoleptic changes during meat storage; therefore, preventing the bacterial growth at interfacial level of meat surface-packaging film is of paramount importance to both producers and consumers groups of these products. Kim et al. (2011) reported that chitosan films with high viscosity (100 and 200 mPa s) did not exhibit any antimicrobial effect against *Escherichia coli* and *Salmonella typhimurium*; although, those were effective against *Listeria monocytogenes*. No, Park, Lee, and Meyers (2002) reported that the growth of *E. coli* was inhibited more efficiently by chitosan having high molecular weight compared with low molecular weight ones. The aim of this research was to prepare nanocomposites deserving favourable thermal properties (compared with synthetic polymers) for the food industry and to display antimicrobial advantages of chitosan–nanocellulose biocomposites, which could be regarded as their superiority over common synthetic polymers. Also, our objective was to evaluate efficiency of chitosan–nanocellulose biocomposites in a real food model system for the first time in order to reveal any possible significant improvements in the product's shelf life as it is in a great demand for the meat industry nowadays.

2. Materials and methods

2.1. Materials

Chitosan powder, having molecular weight of 600–800 kDa, and cellulose nanoparticles, with 20–50 nm diameters, were purchased from Acros Organics Co., Belgium and Asahi Kasei Corp., Japan, respectively. Pure acetic acid and glycerol were obtained from Merck Co., Germany. Culture media of Nutrient Broth, Brain Heart Infusion (BHI), de Man Rogosa Sharpe (MRS) Broth and Agar and Bacto-Peptone were purchased from Merck Co., Germany and Hi Media Co., India, respectively. *E. coli* PTCC 1399, *Staphylococcus aureus* PTCC 1431 and *Salmonella enteritidis* PTCC 1381 were supplied by Persian Type Culture Collection, Iran.

2.2. Film preparation

Certain amounts of chitosan powder (Table 1) were dissolved in 40 mL aqueous solution (1% v/v) of glacial acetic acid; stirring was conducted at 50 °C and 250 rpm by a heater-stirrer (CB162, Stuart®, UK) for 2 h. In parallel, certain amounts of n-cellulose (Table 1) were added to 20 mL distilled water and dispersed in the same conditions as chitosan dissolution for 2 h. Finally, the latter solution was added to the former one. Glycerol (Table 1) was dispersed in the solution and thorough homogenization occurred in the similar temperature and speed to chitosan dissolution for 24 h. Then, the solution was homogenized with a rotor stator (IKA®T25 digital, Ultra-Turrax®, Germany) at 8000 rpm for 15 min and an ultrasound device (TI-H-10, Elma®, Germany) at 35 kHz and 100% power without heating for 30 min. Deaeration was conducted in a thermostat vacuum oven

(Croydon, Townson & Mercer Ltd., UK) at 600 mmHg for 1 h without heating. The solutions (60 mL) were casted on the centre of glass plates, having 100 cm² surface areas and placing inside the oven at 37 °C; the required time for film forming was 48 h. The dried films were removed from the plates and placed in the oven for two further days to evaporate residual solvents completely and finally were located in hermetic packaging plastics (18 × 14 cm²; Polyeten Co., Iran).

Final film (after overall optimization) was prepared from 1% (w/0.6v) chitosan powder, 0.18% (w/w_{CHT}) n-cellulose and 30% (v/w_{CHT}) glycerol to apply on the surface of ground meat (Dehnad, Emam-Djomeh, Mirzaei, Jafari, & Dadashi, 2014).

2.3. Thermal analysis

Thermal properties of the NCPs were studied by Differential Scanning Calorimetry (DSC 1, Mettler Toledo, Swiss). 10 mg sample was placed in aluminium DSC pans; the device was calibrated against indium as standard. For the measurements, 4 specimens including sample Nos. 1, 3, 5 and 14 were evaluated. Each sample heated by the instrument under an argon atmosphere at 20 mL/min velocity and heating rate of 10 °C/min up to 170 °C, followed by cooling to –10 °C and reheating to 200 °C (Cerqueira, Souza, Teixeira, & Vicente, 2012). Characteristics of melting peaks and glass transition temperatures (*T_g*) were determined on the basis of first and second scans, respectively (Mucha & Pawlak, 2005).

2.4. X-ray diffraction

Samples were analyzed between $2\theta = 5\text{--}45^\circ$ with step increment of $2\theta = 0.02^\circ$ in a Bruker Diffractometer (D8 Advance, Siemens, Germany) using a Cu K α irradiation (40 kV/30 mA) at the wavelength of 1.54 Å (Li et al., 2011). Sample Nos. 2, 4, 6 and 8 were selected to study the effect of different n-cellulose and glycerol levels on microcrystalline structure of NCPs.

2.5. Agar disc diffusion test

E. coli, *S. aureus* and *S. enteritidis* were grown on the nutrient agar slant and kept at 4 °C. To prepare liquid culture of bacteria, a whole loop of each bacterium was cultured into 50 mL BHI sterile medium. Then, cultures of bacterial strains (in BHI) were agitated at 140–150 rpm and 37 °C for 24 h in a shaker incubator (SI50, Stuart®, UK). A dilution series was carried out to meet required bacterial population for seeding, by using sterile distilled water. Agar diffusion method was used for determining antibacterial properties. Films were cut into squares having 10 or 15 mm sides (with special moulds) for *E. coli* and *S. aureus* or *S. enteritidis*, respectively. Film pieces were placed on BHI Agar. Agar plates had been previously seeded with 0.1 mL of an overnight broth culture for indicator strains. Initial number of bacteria was in the range of $10^5\text{--}10^6$ CFU/mL. Bacterial strains were incubated at 37 °C for 24 h (Pranoto, Rakshit, & Solakhe, 2005). Results were reported as ability (–) or lack of ability (+) for bacterial growth in the contact area with nanocomposites.

2.6. Shelf life investigation of ground meat

Efficacy of the optimized antimicrobial films was evaluated by placing films on the upper surface of 60-mm diameter circular slabs (15 mm thickness) of a ground meat sample (100 g) which was purchased from a local market in Gorgan, Iran. The slabs were aseptically prepared and placed in the bottom sections of petri dishes and then completely covered with chitosan–nanocellulose or nylon films. The sets were individually thermo-sealed in oxygen impermeable films (13 × 13 cm²; PakizehPeyk Plastic Industry,

Table 1
RSM Box–Behnken experimental design for independent variables.

Std. no. of treatment	Chitosan percentage (%w/0.6v)	Chitosan concentration (g/60 mL)	n-cellulose percentage (%w/w _{CHT})	n-cellulose concentration (g)	Glycerol percentage (%v/w _{CHT})	Glycerol concentration (mL)
1	1.00	1.00	0	0.0000	60	0.600
2	1.30	1.30	0	0.0000	60	0.780
3	1.00	1.00	2	0.0200	60	0.600
4	1.30	1.30	2	0.0260	60	0.780
5	1.00	1.00	1	0.0100	30	0.300
6	1.30	1.30	1	0.0130	30	0.390
7	1.00	1.00	1	0.0100	90	0.900
8	1.30	1.30	1	0.0130	90	1.170
9	1.15	1.15	0	0.0000	30	0.345
10	1.15	1.15	2	0.0230	30	0.345
11	1.15	1.15	0	0.0000	90	1.035
12	1.15	1.15	2	0.0230	90	1.035
13	1.15	1.15	1	0.0115	60	0.690
14	1.15	1.15	1	0.0115	60	0.690
15	1.15	1.15	1	0.0115	60	0.690

Iran) using a Press-plast machine (ArnikaMana Industrial Group, Iran) and finally stored at 4 or 10 °C for 6 days prior to enumerating bacteria. At the specific intervals (2 days), covered meats were aseptically removed from their packs and total microbial count and pH of samples were determined. pH measurements were carried out using a digital pH metre (Knick pH-Metre 766 Calimatic, Germany) at room temperature. Three different points of each sample were selected and the mean values were reported. At the times of enumeration, a thin layer (0.2–0.3 cm thickness) of each sample including the surface having been received the antimicrobial film was aseptically excised, placed in a 400-mL homogenizing bag along with 90 mL of 0.1% (w/v) Bacto-Peptone, and massaged for 120 s at high speed in a Stomacher 400 laboratory blender (Seward, UK). Appropriate 10-fold dilutions of the homogenate were spread-plated (0.1 mL per plate) onto MRSA for enumeration of lactic acid bacteria (LAB). The plates were subsequently incubated at 25 °C for 72 h before counting colonies (Ouattara, Simard, Piette, Bégin, & Holley, 2000). All microbiological counts were expressed as the log of colony-forming units per mL of homogenized sample (log CFU/mL).

2.7. Statistical analysis

Design-Expert® 6.0.2 software was used to study the influence of above-mentioned variables on different responses. Response Surface Methodology and Box–Behnken design were applied for three independent variables including chitosan, n-cellulose and glycerol at three levels and three replications at central point (Table 1).

3. Results and discussion

3.1. Thermal properties

The results of DSC tests have been listed in Table 2. When treatment Nos. 1 and 3 are compared with each other, it is illustrated that increasing n-cellulose content decreased glass transition temperature. This could be due to an increase in chitosan movement as a result of reaction with n-cellulose layers (Kubies et al., 2006); in other words, addition of nanoparticles (up to 2%) could speed up moving chitosan chains towards their glass transition temperatures, which causes an improvement in polymer functionality of thermo-sealing aspects. Comparison of sample No. 14 with sample No. 3 (and 1) indicates that increasing chitosan from 1 to 1.15% (w/0.6v) and glycerol decreased glass transition temperature. High contents of glycerol resulted in increasing void spaces of polymer matrix and molecular movements, changing physical structure of

the film and decreasing glass transition temperature (Roos & Karel, 1991). Cerqueira et al. (2012), with assistance of FTIR analysis, confirmed that glycerol addition led to more hydrogen bonds in the film context, following by an increase in structural order and accelerating occurrence of glass transition temperature.

Increase in n-cellulose percentage lowered melting point (T_m) of the NCP; therefore, its crystalline structure had been slightly decreased. Attendance of n-cellulose particles resulted in decreasing accumulation of chitosan chains and obtaining final structures with lower crystallinity degrees (Hassan, Hassan, & Oksman, 2011). The lowest rates of melting enthalpy and melting area were observed in sample No. 14 containing the highest level of applied chitosan and glycerol. Increasing glycerol from 30 to 60 (%v/w_{CHT}) culminated in lower melting enthalpy and melting area as it could be observed by comparing sample No. 3 (and 1) with sample No. 5. Melting process was occurred during some temperature-time ranges, observed as decreasing and increasing trends in Fig. 1-a. Peaks for sample Nos. 1 and 5 were higher than that of others, indicating stronger and more complex internal bonds within the samples. Fig. 1-b., which shows glass transition ranges

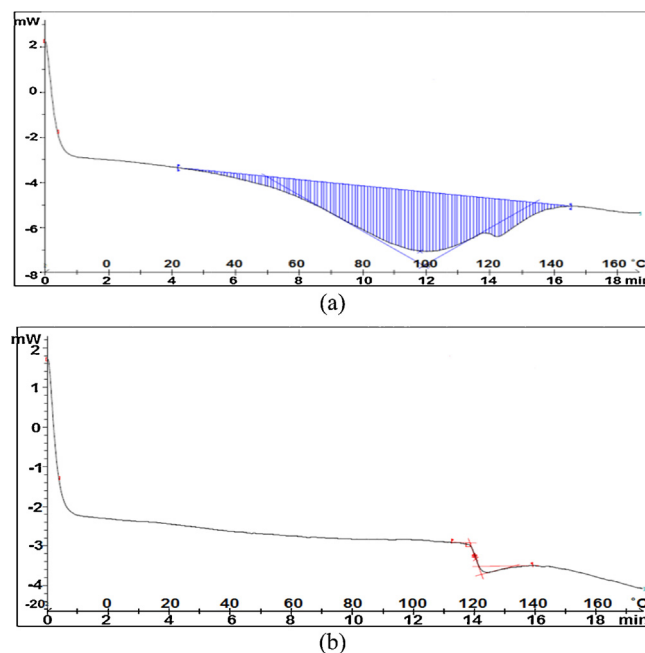


Fig. 1. (a) Melting and (b) glass transition stages for chitosan–nanocellulose bio-composite (sample No. 3) by DSC analysis.

Table 2

Thermal properties of chitosan–nanocellulose biocomposites.

Std. no.	T_g (°C)	T_m (°C)	Enthalpy of melting ($J g^{-1}$)	Peak height of melting (mW)	Calculated area of melting curve (mJ)
1	123.06	98.99	–123.34	3.85	–1221.07
3	119.95	97.73	–88.28	2.64	–882.78
5	121.45	97.87	–121.39	3.53	–1226.09
14	115.88	97.44	–86.38	2.50	–872.41

Table 3

XRD results for chitosan–nanocellulose biocomposites.

Std. no.	d-space (Å)	Angle (2-Theta°)	Peak intensity (count)	Peak intensity (%)	Raw area (Cps $\times$ 2- θ°)	Net area (Cps $\times$ 2- θ°)
2	4.37	20.304	16.4	100	322.7	164.60
4	4.20	21.160	10.2	100	104.3	52.14
6	4.48	19.791	18.2	100	331.7	218.60
8	4.25	20.874	17.0	100	202.8	86.55

of polymers, and Fig. 1-a. indicated attendance of only one peak; in other words, two layers including chitosan matrix and cellulose nanoparticles were merged and united well.

T_g values of the prepared chitosan–nanocellulose biocomposites were higher than those of most synthetic films, implying their suitability as an alternative for synthetic films; for instance, –110, –14, 87, 95 °C have been reported for low density polyethylene, polypropylene, poly(vinyl chloride), and polystyrene, respectively (Kalpakjian & Schmid, 2008). Although T_m values of the NCPs were lower than common synthetic films to some extent, those values were in a reasonable range of applicable films (Kalpakjian & Schmid, 2008); besides, they are particularly attractive for packaging industry because of their better recycling capabilities.

3.2. XRD results

Chitosan films exhibited a relatively broad peak at around $2\theta = 20^\circ$, which shows their semi-amorphous nature. Applied n-cellulose had a crystalline structure, previously approved by Dadashi, Mousavi, Emam-Djomeh, and Oromiehie (2012) who reported a strong peak at $2\theta = 30.26^\circ$ for applied n-cellulose. When NCP No. 2 is compared with sample No. 4, it could be found that addition of n-cellulose particles displaced the position of chitosan peak to the higher angles (near to the range of n-cellulose peak) but decreased the peak intensity and the gaps among the sheets (Table 3). XRD pattern of chitosan films did not show any significant changes in peak position due to n-cellulose addition; in other words, n-cellulose peak was completely disappeared, which strengthens the configuration probability of intercalated or exfoliated structures into the prepared NCPs. Comparison of the results for sample Nos. 6 and 8 demonstrates that decreasing glycerol increased peak intensity and spaces among the sheets, in agreement with the larger area below peak No. 6 (Fig. 2.).

3.3. Antimicrobial assay

The results of agar disc diffusion tests have been listed in Table 4. Chitosan–nanocellulose NCPs exhibited lethal effects against both gram-positive (*S. aureus*) and gram-negative (*E. coli* and *S. enteritidis*) bacteria through their contact areas. In this research, 1–1.3% (w/0.6v) chitosan completely restrained microbial growth; similarly, Park, Marsh, and Dawson (2010) reported that incorporation of 0.3 and 0.7%(w/w) chitosan into LDPE could not decrease bacterial population of *E. coli*, *L. monocytogenes* and *S. enteritidis* to the zero while incorporation of 1.4–2.1%(w/w) chitosan completely prohibited attendance of those bacteria on the medium in contact areas.

Chitosan–nanocellulose NCPs represented antimicrobial effects only to the extents of their contact areas; in other words, antimicrobial effect of the NCP, measured by this test, was independent on chitosan, n-cellulose and glycerol concentrations. It deserves to point out that antimicrobial agents, like chitosan, always need water to be activated on the medium; completely dried samples cannot release their saved energies (in their chemical bonds) on the medium in order to start appropriate reactions (Kong, Chen, Xing, & Park, 2010). In the current research, while increasing n-cellulose and glycerol levels increased water solubility of the NCPs (Dehnad et al., 2014), they exhibited no increasing or decreasing effects on distribution of antimicrobial effects in chitosan films (in this special test; Fig. 3.).

3.4. Application of NCP on the product

Application of chitosan–nanocellulose NCPs on the ground meat decreased LAB population by approximately 1.3 and 3.1 logarithmic cycles at 3 °C and 25 °C compared with nylon packaged samples, respectively (Table 5). It has been highly qualified that antimicrobial activity of chitosan films are firmly dependent on the degree of their hydrophilic/hydrophobic nature (Kong et al., 2010); therefore, n-cellulose particles might play a role in implementing high performance of chitosan film against those bacterial populations because increasing n-cellulose level could significantly increase solubility in water of chitosan film. Our results implied that releasing acetic acid, known as an inhibitory agent of microorganism growth (Fernandez-Saiz, Soler, Lagaron, & Ocio, 2010), from

Table 4

Results of agar disc diffusion tests for chitosan–nanocellulose biocomposites.

Std. no.	<i>S. aureus</i>	<i>E. coli</i>	<i>S. enteritidis</i>
1	+	+	nd ^b
2	+	+	+
3	+	+	nd
4	+	+	+
5	+	+	nd
6	+	+	+
7	+	+	nd
8	+	+	+
9	+	+	nd
10	+	+	nd
11	+	+	nd
12	+	+	nd
13	+	+	nd
14	+	+	nd
15	+	+	+

^a + = No bacterial growth in contact area.^b nd = Non-detected.

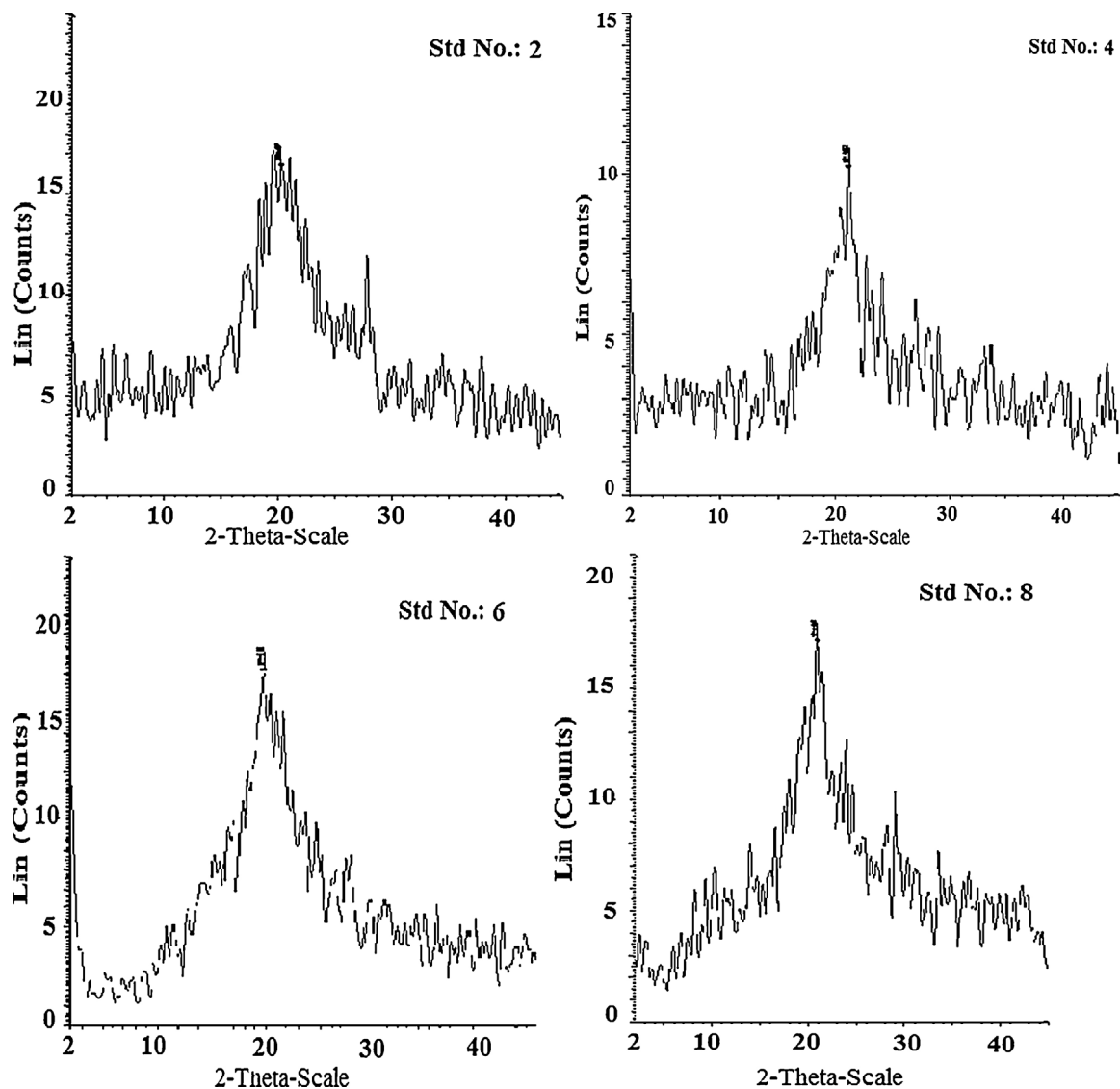


Fig. 2. XRD photographs for chitosan–nanocellulose biocomposite (Nos. 2, 4, 6 and 8).

polymer matrix could reasonably occur faster at higher temperatures of storage rather than the lower ones. Ouattara et al. (2000) reported that application of chitosan film (2% (w/v)) on the pastрами at 3 °C decreased LAB population insignificantly (0.6 logarithmic cycles) compared with control sample after 21 days and reported that those films had no or little effects on LAB whether inoculated or indigenous. Park, Marsh, and Dawson (2010) incorporated 1–8% chitosan into LDPE films; they observed that there were no significant differences in log values of total viable count between control and chitosan-incorporated LDPE films and no antimicrobial activity was observed for chitosan incorporated films during storage time.

Decreasing bacterial population at refrigerator temperature was sharper at the beginning of course than the end of period; indeed, releasing acetic acid from polymer matrix was fast at the beginning because of ion gradient between internal and external spaces of polymer matrix although the released amount decreased by gradual development of the reaction. The highest inhibitory effects of NCPs were achieved after 4 and 6 days of storage at 25 °C, equal to 2.25 and 3.08 logarithmic cycles, respectively; pH analyses indicated that NCPs could maintain pH of related samples, stored at 25 °C for 4 and 6 days, lower than control samples (0.12 and 0.2, respectively). The pH decline approved the theory of inhibitory

Table 5
Inhibitory effect of chitosan–nanocellulose nanocomposite against LAB initially present on ground meat samples in terms of bacterial population (Log_{10} CFU/mL) and pH values of the samples after given storage conditions.

Bacterium	Storage conditions							
	3 °C				25 °C			
	0 (days)	2 (days)	4 (days)	6 (days)	0 (days)	2 (days)	4 (days)	6 (days)
LAB	2.48	4.00 [5.30] ^a	4.68 [5.31]	3.94 [4.99]	2.48	5.04 [5.02]	3.07 [5.32]	2.36 [5.44]
pH	5.79	5.88 [5.70]	5.79 [5.60]	5.71 [5.51]	5.79	5.32 [5.20]	5.32 [5.44]	5.98 [6.18]

^a The values of bacterial population for nylon packaged (control) samples have been presented in the brackets.

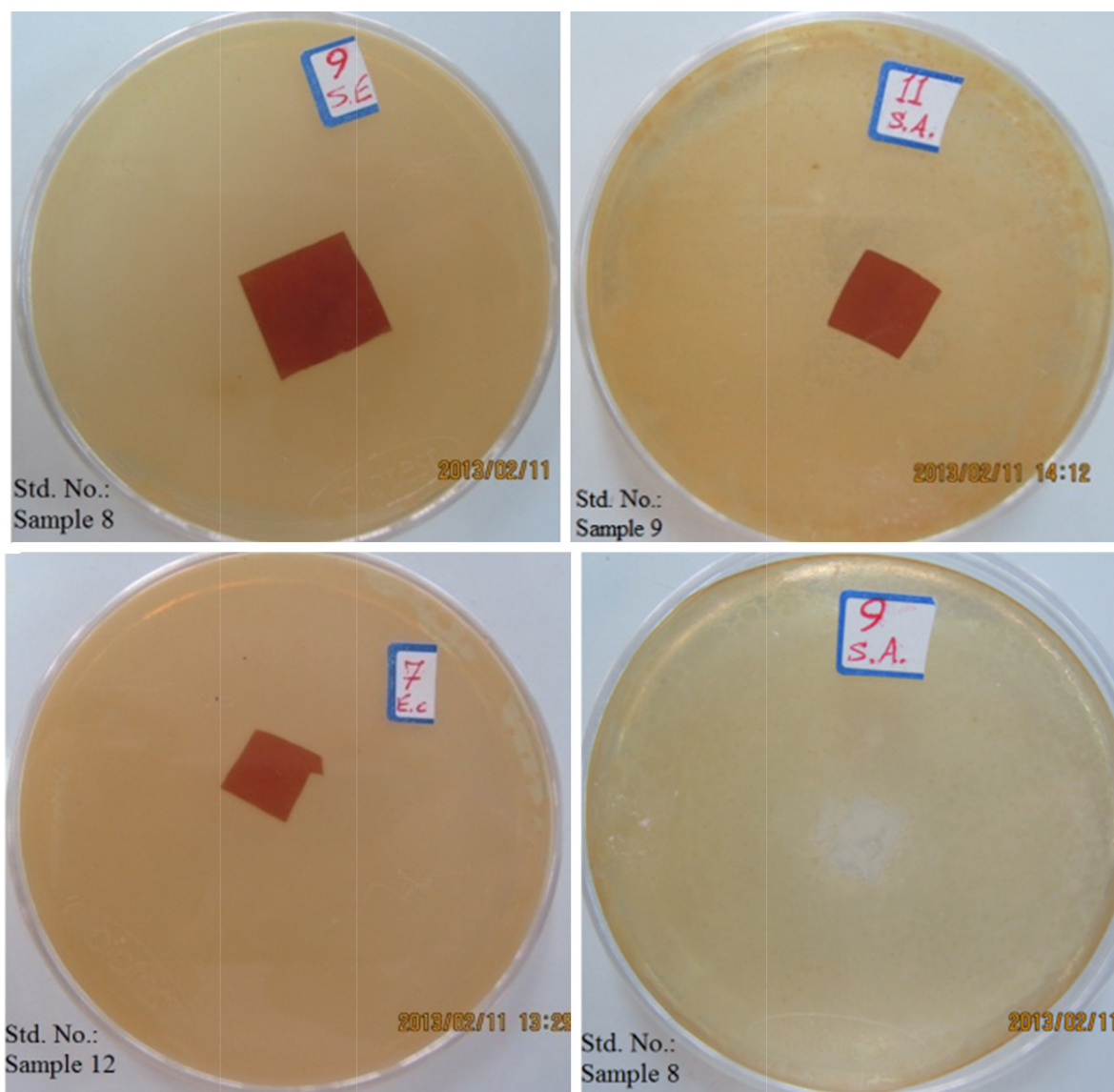


Fig. 3. Nanocomposite Nos. 8, 9 and 12 on the inoculated media with *S. enteritidis*, *S. aureus* and *E. coli* (respectively); the last image (right) shows the beneath area of NCP No. 8, applied on the inoculated medium with *S. aureus* and exhibited non-growth area.

effect of released acetic acid (from polymer matrix) against microbial growth. Similarly, the lowest rate of inhibitory effect was related to 2 days stored meat; pH test confirmed that edible films could not make any positive changes (compared with control samples) in pH of the product during 2 days.

4. Conclusion

Three main drawbacks of bio-nanocomposites are their poor thermal stability in comparison with common synthetic films, unsatisfactory dispersion rate (nanoparticles into matrices) and lack of enough knowledge about their probable practical antimicrobial properties; in contrast, chitosan–nanocellulose NCP deserved “suitable bio-nanocomposite” title. T_g values of prepared chitosan–nanocellulose bio-composites were higher than those of the most synthetic films; besides, they were particularly attractive for recycling purposes as relating thermal aspects. XRD pattern of NCP exhibited that n-cellulose peak disappeared completely after addition to and homogenizing by chitosan matrix, which strengthens the configuring probability of intercalated or exfoliated

structures into the NCPs. Finally, NCP indicated excellent inhibitory effects against gram-positive and gram-negative bacteria, promising for food packaging; application of chitosan–nanocellulose NCP on the ground meat decreased lactic acid bacteria population up to 3.1 logarithmic cycles (compared with nylon packaged sample) at 25 °C during 6 days of storage.

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References

- Azerado, H. M. C., Mattoso, L. H. C., Avena-Bustillos, R. J., Filho, G. C., Munford, M. L., Wood, D., & McHugh, T. H. (2010). Nanocellulose reinforced chitosan composite films as affected by nanofiller loading and plasticizer content. *Journal of Food Science*, 75, N1–N7.

- Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., & Vicente, A. A. (2012). Effect of glycerol and corn oil on physicochemical properties of polysaccharide films – A comparative study. *Food Hydrocolloids*, 27, 175–184.
- Dadashi, S. (2011). *The effect of adding nanoparticles on mechanical and thermal properties of PLA films. (dissertation)*. Karaj: University of Tehran, University College of Agriculture & Natural Resources, Faculty of Agronomy Engineering & Technology.
- Dadashi, S., Mousavi, S. M., Emam-Djomeh, Z., & Oromiehie, A. (2012). Films based on poly(lactic acid) biopolymer: Effect of clay and cellulosic nanoparticles on their physical, mechanical and structural properties. *Iranian Journal of Polymer Science and Technology*, 25, 127–136.
- De Mesquita, J. P., Donnici, C. L., Teixeira, I. F., & Pereira, F. V. (2012). Bio-based nanocomposites obtained through covalent linkage between chitosan and cellulose nanocrystals. *Carbohydrate Polymers*, 90, 210–217.
- Dehnad, D., Emam-Djomeh, Z., Mirzaei, H., Jafari, S.-M., & Dadashi, S. (2014). Optimization of physical and mechanical properties for chitosan–nanocellulose biocomposites. *Carbohydrate Polymers*, 105, 222–228.
- Fernandes, S. C. M., Freire, C. S. R., Silvestre, A. J. D., Pascoal Neto, C., Gandini, A., Berglund, L. A., & Salmen, L. (2010). Transparent chitosan films reinforced with a high content of nanofibrillated cellulose. *Carbohydrate Polymers*, 81, 394–401.
- Fernandes, S. C. M., Oliveira, L., Freire, C. S. R., Silvestre, A. J. D., Neto, C. P., Gandini, A., & Desbrieres, J. (2009). Novel transparent nanocomposite films based on chitosan and bacterial cellulose. *Green Chemistry*, 11, 2023–2029.
- Fernandez-Saiz, P., Soler, C., Lagaron, J. M., & Ocio, M. J. (2010). Effects of chitosan films on the growth of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. in laboratory media and in fish soup. *International Journal of Food Microbiology*, 137, 287–294.
- Güçbilmez, Ç. M., Yemenicioğlu, A., & Arslanoğlu, A. (2007). Antimicrobial and antioxidant activity of edible zein films incorporated with lysozyme, albumin proteins and disodium EDTA. *Food Research International*, 40, 80–91.
- Hansson, S., Trouillet, V., Tischer, T., Goldmann, A. S., Carlmark, A., Barner-Kowollik, C., & Malmstrom, E. (2013). Grafting efficiency of synthetic polymers onto biomaterials: A comparative study of grafting-from versus grafting-to. *Biomacromolecules*, 14, 64–74.
- Hassan, M., Hassan, E., & Oksman, K. (2011). Effect of pretreatment of bagasse fibers on the properties of chitosan/microfibrillated cellulose nanocomposites. *Journal of Materials Science*, 46, 1732–1740.
- Kalpajian, S., & Schmid, S. R. (2008). *Manufacturing Processes for Engineering Materials* (5th ed.). Pearson Education.
- Khan, A., Khan, R. A., Salmieri, S., Le Tien, C., Riedl, B., Bouchard, J., Chauve, G., Tan, V., Kamal, M. R., & Lacroix, M. (2012). Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films. *Carbohydrate Polymers*, 90, 1601–1608.
- Kim, K. W., Min, B. J., Kim, Y.-T., Kimmel, R. M., Cooksey, K., & Park, S. I. (2011). Antimicrobial activity against foodborne pathogens of chitosan biopolymer films of different molecular weights. *LWT – Food Science and Technology*, 44, 565–569.
- Klemm, D., Heublein, B., & Fink, H. P. (2005). Cellulose: Fascinating biopolymer and sustainable raw material. *Angewandte Chemie International Edition*, 44, 3358–3393.
- Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology*, 144, 51–63.
- Kubies, D., Scudla, J., Puffr, R., Sikora, A., Baldrian, J., Kovarova, J., Slouf, M., & Rypacek, F. (2006). Structure and mechanical properties of poly(L-lactide)/layered silicate nanocomposites. *European Polymer Journal*, 42, 888–899.
- Li, B., Li, J., Xia, J., Kennedy, J. F., Yie, X., & Liu, T. G. (2011). Effect of gamma irradiation on the condensed state structure and mechanical properties of konjac glucomannan/chitosan blend films. *Carbohydrate Polymers*, 83, 44–51.
- Mayachiew, P., Devahastin, S., Mackey, B. M., & Niranjan, K. (2010). Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract. *Food Research International*, 43, 125–132.
- Mucha, M., & Pawlak, A. (2005). Thermal analysis of chitosan and its blends. *Thermochimica Acta*, 427, 69–76.
- Muzzarelli, R. A. A. (2012). Nanochitins and nanochitosans, paving the way to eco-friendly and energy-saving exploitation of marine resources. *Polymer Science: A Comprehensive Reference*, 10, 153–164.
- Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, 87, 995–1012.
- No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*, 74, 65–72.
- Quattara, B., Simard, R. E., Piette, G., Bégin, A., & Holley, R. A. (2000). Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *International Journal of Food Microbiology*, 62, 139–148.
- Park, S. I., Marsh, K. S., & Dawson, P. (2010). Application of chitosan-incorporated LDPE film to sliced fresh red meats for shelf life extension. *Meat Science*, 85, 493–499.
- Petersson, L., & Oksman, K. (2006). Biopolymer based nanocomposites: Comparing layered silicates and microcrystalline cellulose as nanoreinforcement. *Composite Science and Technology*, 66, 2187–2196.
- Pranoto, Y., Rakshit, S. K., & Salokhe, V. M. (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT – Food Science and Technology*, 38, 859–865.
- Roos, Y., & Karel, M. (1991). Plasticizing effect of water of thermal the behaviour and crystallization of amorphous food models. *Journal of Food Science*, 56, 38–43.
- Tome, L. C., Fernandes, S. C. M., Perez, D. S., Sadocco, P., Silvestre, A. J. D., Neto, C. P., Marrucho, I. M., & Freire, C. S. R. (2013). The role of nanocellulose fibers, starch and chitosan on multipolysaccharide based films. *Cellulose*, 20, 1807–1818.
- Vu, K. D., Hollingsworth, R. G., Leroux, E., Salmieri, S., & Lacroix, M. (2011). Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries. *Food Research International*, 44, 198–203.