Chitosan Oligomers and Related Nanoparticles as Environmentally Friendly Wood Preservatives

Laya Khademibami,* Dragica Jeremic, Rubin Shmulsky, and H. Michael Barnes

The efficacy of chitosan oligomers and related nanoparticles as environmentally friendly wood protection agents was evaluated in this study. Commercially sourced low-molecular weight chitosan was depolymerized using sodium nitrite. Evaluation of depolymerized chitosan to the nano level by thin layer chromatography confirmed acceptable results for obtaining a degree of polymerization of four. Then, oligomers were modified to form quaternized chitosan oligomers. Both quaternized and non-quaternized oligomers were mixed with tripolyphosphate (TPP) to form nano-chitosan-TPP particles *via* an ionic gelation method. Southern pine wood samples were treated with different chitosan-based solutions and suspensions under a vacuum impregnation process. The mass and volume of the treated samples were calculated before and after treatments to evaluate bulking. The mass loss after leaching of the treated wood samples was calculated. The mass and volume gain results indicated that quaternized nano-chitosan-TPP treated samples had more mass and volume gain after treatment in comparison with non-quaternized nano-chitosan-TPP-treated and control samples. The mass loss results revealed that mass loss increased in quaternized nano-chitosan-TPP particles. Although quaternized nano-chitosan particles were positively charged, they could not fix to the cell walls and became leached out. Therefore, these nanoparticles can likely be used as wood preservatives in non-leaching applications.

Keywords: Nano-chitosan particles; Leaching; Bulking; Southern yellow pine; Wood preservatives

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INTRODUCTION

For decades, wood scientists have been developing technologies and improving the properties of wood to increase its sustainability. Wood preservation and wood modification are solutions for increasing wood durability. Markets for chemically modified wood and heat-treated modified wood are growing. Thus, there is an ongoing need to improve wood preservation technology. Several factors are involved in choosing a suitable preservative, which include a long-term protection, stability, potential effects on wood strength, cost, and safety of the treated wood for human and environmental exposure (Ozdemir *et al.* 2014). Currently, the use of widely accepted preservatives plays a vital role in the wood industry. Due to environmental concerns and constraints on the use of various current heavy metal preservatives for humans and the environment, there is a new trend in wood preservation to explore more environmentally benign chemicals (Treu *et al*. 2009). Therefore, developing and using wood preservatives that are safe for humans and the environment that reduce hazardous wastes are vital needs.

Recently, chitosan has been gaining attention due to its non-toxic nature, antimicrobial activity, biodegradability, and biocompatible abilities (Xu *et al*. 2010; Kumar *et al.* 2017; Ikono *et al.* 2019). Chitosan has a chemical structure similar to cellulose and is the next most widely available natural polymer after the main components of wood

(Kumar 2000). Chitosan, as a natural polymer, is derived from chitin, which is a biopolymer and polysaccharide component of crustacean shells such as shrimps, crabs, and lobsters. Chitosan is generally water-soluble under acidic conditions, while chitin is insoluble in water (Eikenes *et al*. 2005). Antifungal activity of chitosan against wood-decaying fungi and forest pathogens has been previously reported (Chittenden *et al*. 2003; Alfredsen *et al*. 2004; Eikenes *et al*. 2005). Amino groups in chitosan structure exhibit antimicrobial activities (Seong *et al*. 1999; Junior 2016). Chitosan has varying average molecular weights. Different molecular weights of chitosan have been studied for wood treatment (Larnoy *et al*. 2006a).

Regardless of different molecular weights (high and low) of chitosan, the penetrability of chitosan into wood cells is associated with concentration. Chitosan with lower concentrations (and their associated lower viscosities) exhibits a greater fixation as well as increased penetration into wood cell walls (Larnoy *et al.* 2006b). Higher concentrations (> 4%) of chitosan have better performance for suitable antifungal activity (Hussain *et al.* 2012). Thus, a nano-polymer form of chitosan should be used to maximize its bonding to wood and to optimize antimicrobial activities. Chitosan can be easily refined toward the nanoscale by chemical processing to form nano-sized particles. This paper is the first of two evaluating the efficacy of nano-chitosan as an environmentally friendly wood preservative agent in indoor and outdoor applications. This paper covers the preparation of nano-chitosan-tripolyphosphate (TPP) particles, bulking of treated samples, and the leachability of the preservative system. A second paper will discuss bio-efficacy.

EXPERIMENTAL

Materials

The methodology of this study focused on two main parts, the preparation of nano-chitosan-TPP particles as wood preservatives and the treatment of wood samples with nano-chitosan oligomers and N,N,N-trimethylchitosan (TMC) oligomers alone or in combination with TPP.

Preparation of Nano-chitosan-TPP Wood Preservatives

Depolymerization of chitosan to oligomers

Commercially available low-molecular weight (LMW) chitosan (50 kDa to 190 kDa) with a degree of deacetylation greater than 75% was used (Sigma-Aldrich, St. Louis, MO, USA). Chitosan was depolymerized by nitrous acid into oligomers with a degree of polymerization (DP = 4) according to the method described by Hussain *et al*. (2012). Briefly, 40 g of chitosan was stirred into 0.1 M aqueous HCL (800 mL) and was heated to 50 °C for 1 h. Then, an applicable amount of $NaNO_2$ was dissolved in 32 mL of water (16.56 g obtaining 40 g of DP = 4). The $NaNO_2$ was added dropwise to the chitosan solution and stirred for 30 min by lowering the viscosity of the mixture. The reaction mixture was stirred at 50 °C for 5 h. Then, 1 M NaOH was added to neutralize the solution (pH 7 to pH 7.5). The solution was filtered through Teflon filter paper. The filtrate was participated by methanol 1/1 (v/v). Finally, the supernatant of methanol was precipitated with acetone 1/1 (v/v). Chitosan oligomers were previously obtained by precipitation in acetone after depolymerization by nitrous acid (Furusaki *et al.* 1995; Cha *et al.* 2000). Therefore, the precipitate of acetone in current study was understood to consist mainly of chitosan oligomers (DP=4).

Analysis of chitosan oligomers by thin layer chromatography (TLC)

Chitosan oligomers were qualitatively analyzed by thin layer chromatography (TLC) using a silica gel plate (Merck 60. GF-254; Sigma-Aldrich, St. Louis, MO, USA) and compared to the standard chitotetraose (tetramer, DP = 4). The standard chitotetraose, 4 HCl was purchased from Carbosynth, Co. (Compton, England). Both chitosan oligomers and standard were dissolved in 50% methanol and loaded on TLC plate with three different volumes (1 μL, 2 μL, and 3 μL). The plate was placed into the solvent system of n-propanol-30% ammonia water (2:1 v/v) for 24 h. Finally, spots were visualized by spraying (0.1% ninhydrin into n-butanol-saturated water) and then baked in an oven at 110 °C for 10 min according to the method described by Choi *et al.* (2004).

Quaternization of chitosan oligomers (Preparation of N,N,N-trimethylchitosan)

Chitosan oligomers from the depolymerization process were dissolved in 1% w/w aqueous acetic acid solution according to the procedure described by Bordenave *et al*. (2008). This process was performed in two separate steps. In the first step, formaldehyde (3 mol formaldehyde per mol of chitosan-free amine groups) was added to chitosan oligomers. Then, the solution was stirred at 500 rpm at room temperature for 30 min before adding $NaBH_4$ (1.5 mol of $NaBH_4$ per mol of formaldehyde), and then the solution was stirred for 1 h. The pH was adjusted to 10 using 1 M NaOH. The unreacted products were then removed by soaking the dialysis tubes (Biotech CE Dialysis Tubing, New Brunswick, NJ, USA) 0.1 kDa to 0.5 kDa molecular weight cut-off (MWCO) in water, and then unreacted products were removed by soaking an ethanol/diethyl ether solution (80/20 v/v) for 48 h. In the second step, formed N-methyl chitosan was dispersed in N-methyl-2-pyrrolidinone with Na_2SO_4 (0.1 M) at 60 °C and later stirred at 1200 rpm for 1 h. Approximately 15% w/w aqueous solution of NaOH containing $(CH_3O)_2SO_2$ (1.5 mol of dimethyl sulfate per mole of free amine groups of the extracted and dried N-methyl chitosan) was added. The mixture was stirred at 500 rpm at 60 °C for 6 h. Finally, formed N,N,N-trimethylchitosan was precipitated using acetone, washed with acetone, and dried under vacuum at room temperature above P_2O_5. The N,N,N-trimethylchitosan (quaternized chitosan oligomers or TMC) was considered a final product for this step.

Preparation of nano-chitosan-TPP particles

Nano-chitosan-TPP particles for non-quaternized oligomers and TMC samples were prepared according to the procedure described by Huang *et al* (2009). Briefly, chitosan oligomers were dissolved in 2% acetic acid and stirred for 30 min. Then, 85% sodium tripolyphosphate (TPP) (Acros Organics, Geel, Belgium) was dissolved in double-distilled water. In the meantime, non-quaternized oligomers and TMC solutions were stirred at room temperature; then TPP solution was added dropwise at a ratio of 2:5 (TPP: chitosan, 4.8:12, w/w) for 2 h. This mass ratio was determined to maximize the number of nanoparticles (Huang *et al*. 2009). After stirring for 2 h, the solution became opalescent. It was then aliquoted into 50-mL centrifuge bottles and centrifuged at 3000 rpm for 10 min. The precipitated nanoparticles were rinsed with distilled water and air-dried to increase concentration. The final concentrations for chitosan and TPP were 12% and 4.8%, respectively.

Analysis of nano-chitosan oligomers, quaternized nano-chitosan oligomers, and nano chitosan-TPP particles

Nano-chitosan oligomers, quaternized nano-chitosan oligomers, and nano chitosan-TPP particles were characterized by Fourier transform infrared spectroscopy (FTIR) (PerkinElmer Spectrum Two Spectrometer: PerkinElmer, Waltham, MA, USA). The FTIR analysis was performed between 450 cm^{-1} to 4000 cm^{-1} using the attenuated total reflectance spectroscopy method (ATR-FTIR). The samples were placed on the ATR crystal prism and 16 scans were attained at 2 cm^{-1} resolution.

Preparation of Wood Samples and Impregnation of Wood Samples with Nano-chitosan-TPP Particles

A piece of defect-free southern yellow pine (*Pinus* spp.) sapwood (Sustainable Bioproducts Department, Starkville, MS, USA) with a density of 0.31 g/cm^3 (oven-dry mass and volume) was cut to make samples with dimensions of 1.4 cm × 1.4 cm × 1.4 cm (l × r × t) from two adjacent (end matched) wood sticks. Samples were dried to a constant weight at 50 °C. Dry dimensions and mass of all wood samples were measured using a digital caliper and balance that was accurate to 0.1 mg (Precision Balance ME103TE/00; Mettler Toledo, Columbus, OH, USA).

Wood samples were vacuum (> 28 mmHg) impregnated with an 12% aqueous solution of nano-chitosan particles for 20 min. To increase reactivity of wood and promote binding of nano-chitosan-TPP particles to wood, pine wood samples were enzymatically and chemically modified. Chemical modification of wood was performed by acetic acid, and enzymatic modification was obtained through a laccase-mediator system (LMS). Laccase is a copper-containing oxidase enzyme that is found in plants, fungi, and microorganisms. In the presence of the mediator, rates of reactions of laccase can be enhanced. Hydroquinone (HQ), 1-hydroxybenzotriazole (HBT), and 2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) are three common mediators, which have been used with laccase as substrates (Morozova *et al*. 2007). To determine the best mediator for laccase in this research, compression strength testing of wood samples treated with LMS was performed. Undried samples were performed by a compression instrument designed and manufactured at Mississippi State's Forest Products Laboratory. The results of compression strength testing indicated that HQ was the best among the three mediators that were considered (Table 1).

Overall, 17 treatments with 35 replicates per treatment group were used in this study. The treatments, including controls and nano-chitosan treatments, are listed and described in Table 2. The control treatments were a positive control without TPP and negative control with only 1% acetic acid.

Table 1. Compression Strength Results to Determine the Best Mediator for LMS

Mediator	Compression LSmean (g/mm^2)
HQ	171.63
HBT	129.19
ABTS	151.16

Table 2. Wood Treatments

		Main Treatments	Positive Control	Negative Control
Wood Modification	Chemical	1: Nano-chitosan with TPP[1] in 1% acetic acid	2: Nano-chitosan without TPP in 1% acetic acid	9: 1% acetic acid
		3: Nano-chitosan with TPP in 0.1 mol acetic acid + 0.2 mol NaCl	4: Nano-chitosan without TPP in 0.1 mol acetic acid + 0.2 mol NaCl	
		10: Quaternized nano-chitosan with TPP in 1% acetic acid	11: Quaternized nano-chitosan without TPP in 1% acetic acid	
		12: Quaternized nano-chitosan with TPP in 0.1 mol acetic acid + 0.2 mol NaCl	13: Quaternized nano-chitosan without TPP in 0.1 mol acetic acid + 0.2 mol NaCl	
	Enzymatic	5: Nano-chitosan with TPP in 1% acetic acid + laccase[2] (1 mg/mL) + HQ[3] (10 mmol) before[4]	6: Nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	
		7: Nano-chitosan with TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously[5]	8: Nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously	
		14: Quaternized nano-chitosan with TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	15: Quaternized nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) before	
		16: Quaternized nano-chitosan with TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously	17: Quaternized nano-chitosan without TPP in 1% acetic acid + laccase (1 mg/mL) + HQ (10 mmol) simultaneously	

[1]TPP: Tripolyphosphate, a commercial fire retardant.
[2]Laccase: copper-containing oxidase enzymes found in many plants, fungi, and microorganisms.
[3]HQ: Hydroquinone, mediator of laccase modification. A mediator is used to oxidize and subsequently increase reactivity of wood and promote binding of chitosan to wood.
[4]Before preservative treatment: first, treat wood with laccase and hydroquinone. Then, treat wood with nano-chitosan particles.
[5]Simultaneously: Treat wood with laccase + hydroquinone and nano-chitosan particles at the same time.

Bulking and Retention

Retention was calculated using the value for mass gain (Eq. 1),

$$\text{Mass gain} = \frac{m_2 - m_1}{m_1} \times 100\ (\%) \quad (1)$$

where m_2 is the dry mass after treatment (g) and m_1 is the dry mass (g) before treatment. Bulking coefficient is based on volume gain due to treatment (Eq. 2),

$$\text{Volume gain} = \frac{V_2 - V_1}{V_1} \times 100\ (\%) \quad (2)$$

where V_2 is the dry volume after treatment (mm^3) and V_1 is the dry volume (mm^3) before treatment.

Leaching

To evaluate the efficacy of nano-chitosan for long-term protection, leaching resistance of wood treated with nano-chitosan-TPP particles was evaluated according to AWPA E11-16 (2016). The samples were submerged in deionized water for two weeks, and the water was changed at predetermined standard time intervals during that time. The results of leaching were calculated as a percentage of mass loss according to the following equation,

$$\text{Mass loss} = \frac{m_1 - m_2}{m_1} \times 100\ (\%) \quad (3)$$

where m_1 is the dry mass (g) before leaching and m_2 is the dry mass (g) after leaching.

Statistical Analysis

The experimental design was a completely randomized design, and data for the bulking and leaching tests were analyzed using one-way analysis of variance (ANOVA). The procedure for linear mixed models (PROC GLIMMIX) of SAS 9.4© (SAS Institute Inc, Cary, NC),

$$Y_{ij} = \mu + T_i + E_{ij} \quad (4)$$

where μ is the population mean, Nj is the effect of nano-chitosan-TPP treatments (T = 1 to 17), and E_{ij} is the residual error.

Data for mass and volume gain as well as mass loss data were further tested by contrast analysis using the MIXED procedure of SAS 9.4©. The effects of quaternized nano-chitosan-TPP treatments *vs.* non-quaternized nano-chitosan-TPP treatments, control *vs.* quaternized nano-chitosan-TPP treatments, control *vs.* non-quaternized nano-chitosan treatments, nano-chitosan-TPP particles *vs.* nano-chitosan without TPP particles, and nano-chitosan-TPP particles *vs.* controls were also tested.

RESULTS AND DISCUSSION

Characterization of Chitosan Oligomers by TLC

Figure 1 demonstrates that ninhydrin reacted with free amine groups and produced a deep purple color. This result was confirmed by quantitative determination of free amino groups in chitosan using a method introduced by Curotto and Aros (1993).

Figure 1 also indicates that chitosan oligomers comprised a range of degrees of polymerization (DP_n) that started from 4 when compared to standard chitotetraose (DP_n: 4). Chitotetraose was a standard with DP_n of 4 with 4 amine groups (right part of TLC plate in Fig. 1). The desired DP_n could be achieved with an applicable amount of sodium nitrite.

The TLC analysis revealed that chitosan with a DP_n of 4 was formed through the depolymerization process of LMW chitosan. Cabrera and Cutsem (2005) prepared chitosan oligomers with a degree of polymerization greater than six using acid and enzymatic processes. They used TLC plating to separate methanol soluble chitooligomers. They reported that chitooligomers with degree of polymerization greater than six cannot be separated with this method and the TLC plate only shows chitooligosaccharide with degrees of polymerization less than six.

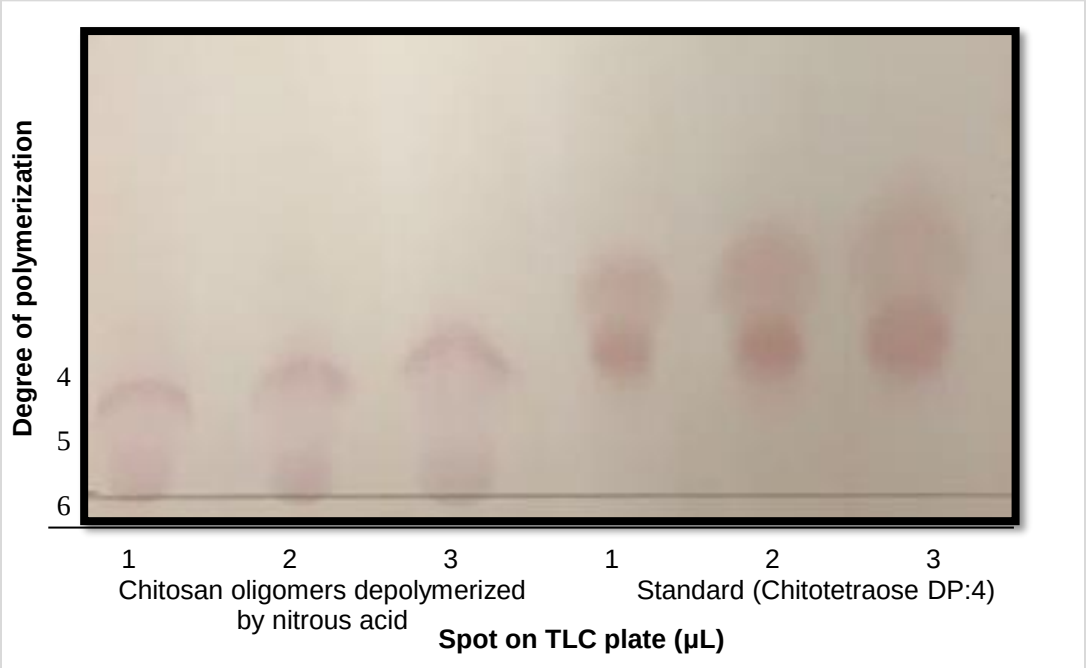

Fig. 1. Thin layer chromatography analysis of chitosan oligomers and chitotetraose

Characterization of Nano-chitosan Oligomers, Quaternized Nano-chitosan Oligomers, and Nano-chitosan-TPP Particles by FTIR

Characterization of nano-chitosan oligomers

The FTIR of chitosan oligomers was obtained after depolymerization of LMW chitosan. These oligomers were then compared to LMW chitosan (Fig. 2). This result indicated that there was a strong chemical structure resemblance between chitosan oligomers and LMW chitosan. However, molecular weight, DP, and degree of acetylation were different between chitosan oligomers and LMW chitosan. In chitosan oligomers, there was a decrease in peaks 1030 cm^{-1} to 990 cm^{-1} attributed to the loss of C-O-C bonds. This indicated that there was a breakdown of chitosan chains. Compared to LMW chitosan, a 1700 cm^{-1} peak subjected to the carbonyl band of an aldehyde group had more intensity in chitosan oligomers, which led to more terminal aldehyde groups in chitosan oligomers.

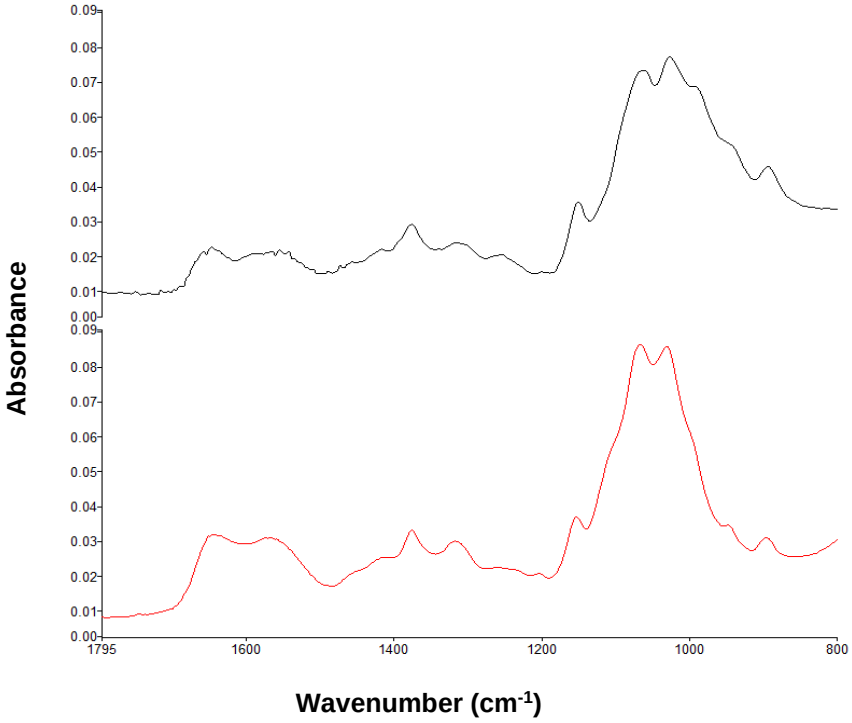

Fig. 2. FTIR of chitosan LMW (black) and nano-chitosan oligomers (red)

Characterization of quaternized nano-chitosan oligomers

The quaternization process was performed after depolymerization and after N,N,N-trimethyl chitosan (TMC) was obtained from chitosan oligomers. Quaternized nano-chitosan is a cationic polyelectrolyte, non-toxic, biocompatible polymer. It is a water-soluble molecule that can be used for nanoparticle production (Xu *et al*. 2003). In Fig. 3, the peak at 1492 cm^{-1} was asymmetrical to a stretch of C-H in methyl groups that was produced in quaternized nano-chitosan oligomers. This peak was new and confirmed that quaternized nano-chitosan oligomers had methyl groups in their structures. Xu *et al*. (2010) used methyl iodide for methylation of low-molecular weight of chitosan. After FTIR was completed, it was found that there was an asymmetrical stretching of the C-H bond in the methyl group at peak 1490 cm^{-1} in TMC. Xu *et al*. (2010) also reported that this peak was because of the highly methylated quaternary of chitosan. The FTIR results of quaternized nano-chitosan oligomers (TMCs) are shown in Fig. 3. In Fig. 3, spectra of quaternized nano-chitosan oligomers were compared to the spectra of non-quaternized nano-chitosan oligomers. Several structural differences were found between these two spectra, which indicated that the derivative from chitosan oligomers exhibited several chemical structural changes. In agreement with the current study, quaternization of high molecular weight (HMW) chitosan was successfully performed by methylation of hydroxyl and amino groups (Bordenave *et al*. 2008). However, asymmetrical stretching of C-H bond in the methyl group was also reported at the 1475 cm^{-1} peak in derivative chitosan (TMC) from HMW chitosan (De Britto *et al*. 2012; Mansur *et al*. 2013). In addition, the peak at 1590 cm^{-1} linked to deformation of N-H in amino groups, which was also previously reported by De Britto *et al*. (2012).

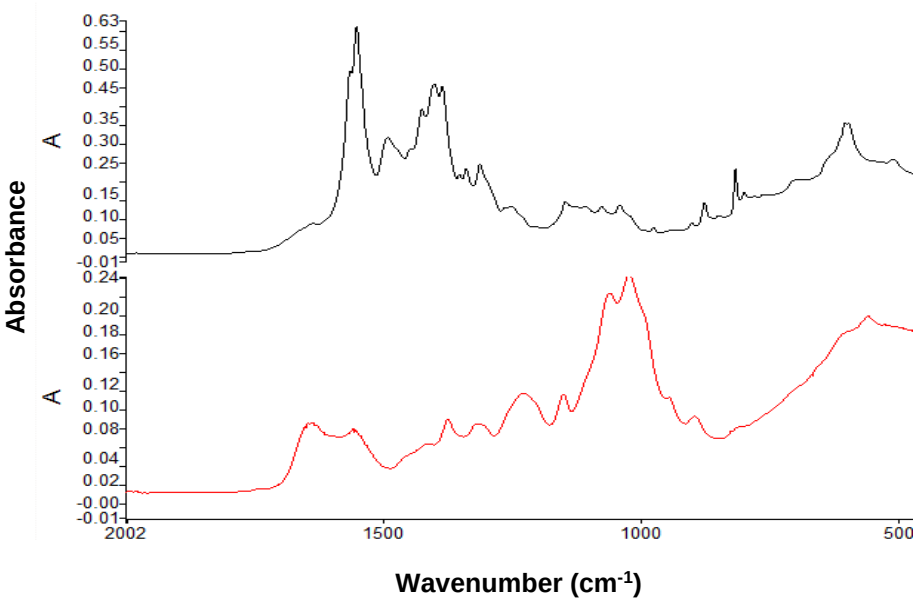

Fig. 3. FTIR of quaternized nano-chitosan oligomers (black) and non-quaternized nano-chitosan oligomers (red)

Characterization of nano-chitosan-TPP and quaternized-nano-chitosan-TPP particles

Based on ionic gelation of chitosan with tripolyphosphate anions, nano-chitosan-TPP particles were prepared (Fig. 4). The formation of non-quaternized nano-chitosan oligomers with TPP was stronger than quaternized nano-chitosan oligomers (trimethylchitosan oligomers, TMC) with TPP. The TPP contained $P_3O_{10}^-$ ions that neutralized amine ions (NH_3^+) in chitosan oligomers (Bhumkar and Pokharkar 2006), but with the TMC process it was more complicated, as TMC had three amine sites, including trimethylated quaternary ($N(CH_3)_3^+$), demethylated ($N(CH_3)_2H^+$), monomethylated ($N(CH_3)\ H_2^+$), and (NH_3^+) sites.

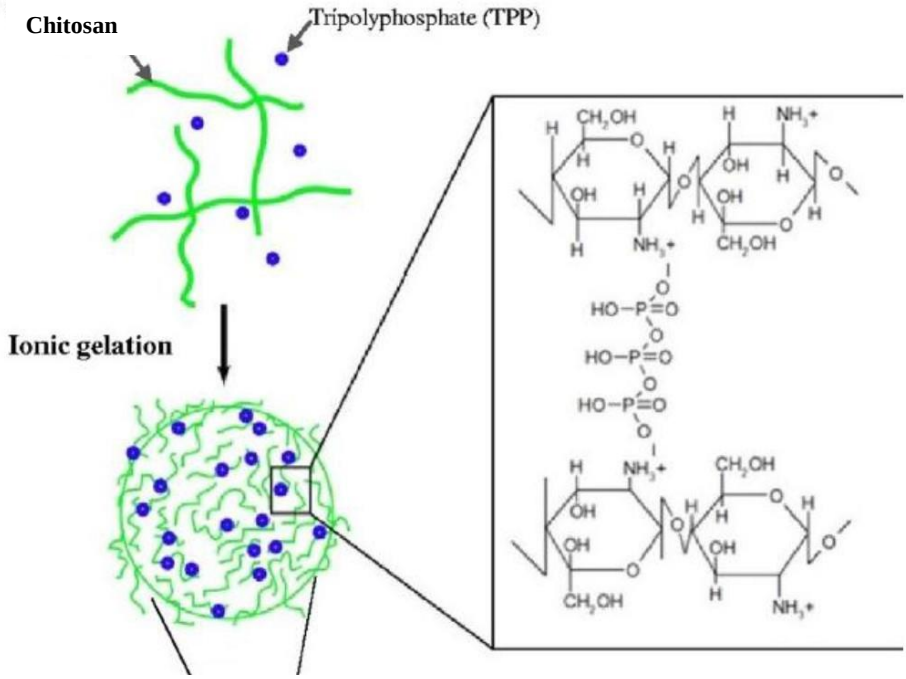

Fig. 4. Ionic gelation of chitosan with tripolyphosphate (TPP) (Ibrahim *et al*. 2017)

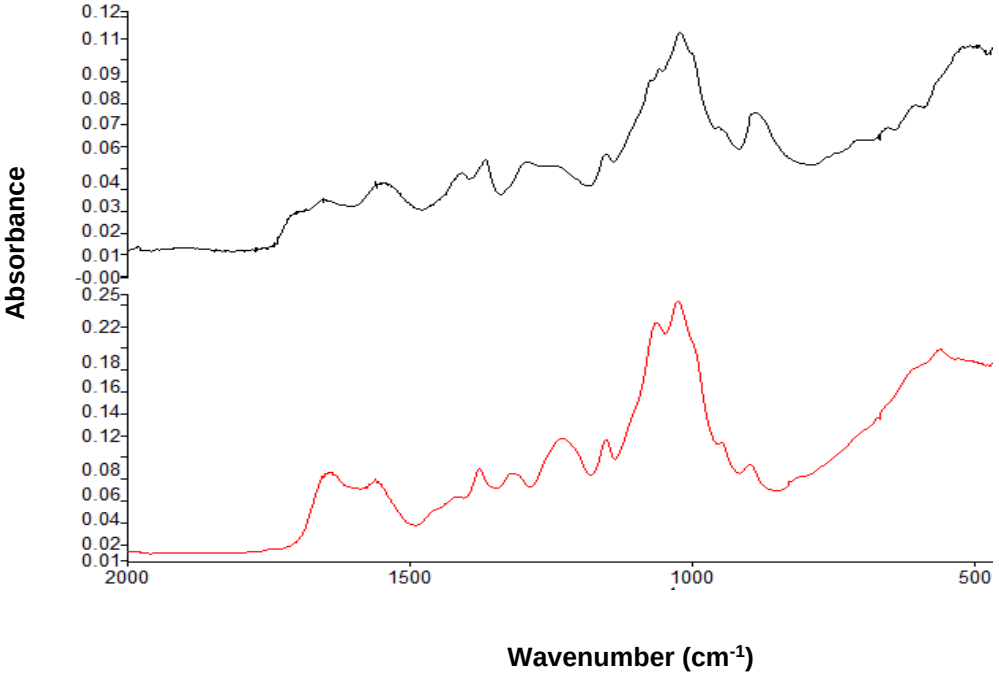

Fig. 5. FTIR of nano-chitosan-TPP particles (black) and nano-chitosan oligomers (red)

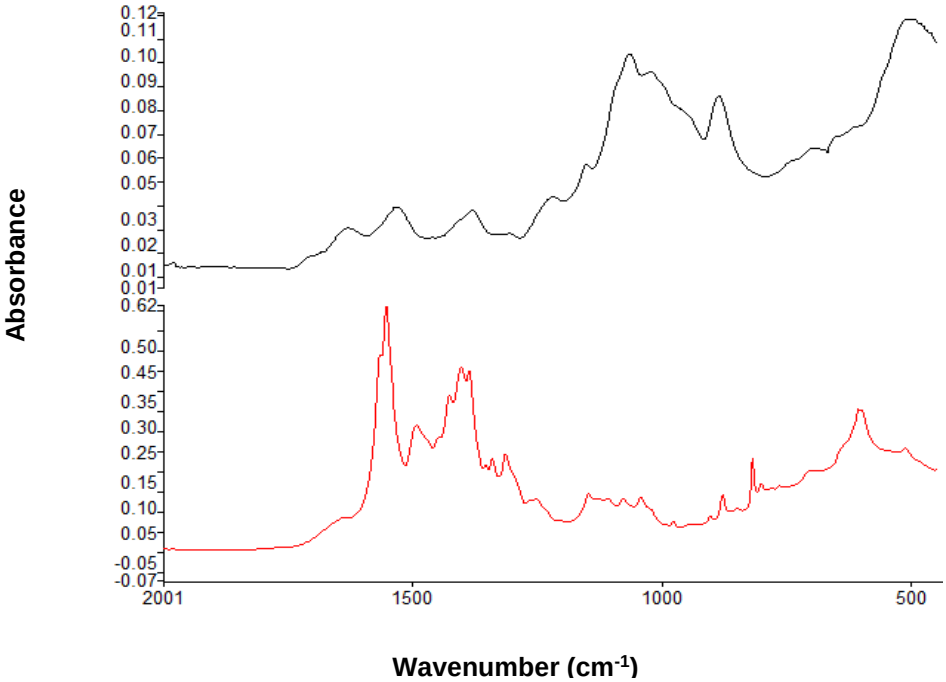

Fig. 6. FTIR of quaternized nano-chitosan-TPP particles (black) and quaternized nano-chitosan (red)

The FTIR spectra for nano-chitosan-TPP particles and quaternized-nano-chitosan-TPP particles are illustrated in Figs. 5 and 6, respectively. The FTIR spectra of nano-chitosan without TPP and nano-chitosan with TPP (Fig. 5) did not show any major differences. However, there were many differences in the two spectra from quaternized nano-chitosan without TPP and quaternized nano-chitosan with TPP (Fig. 6). The 1234 cm^{-1} peak indicated an antisymmetric stretch (PO_2), which indicated that there was likely

an ionic crosslink between both non-quaternized and quaternized nano-chitosan with TPP. Similar to this study, Lasch *et al*. (2002) reported that the 1237 cm^{-1} peak demonstrated an O–P=O antisymmetric stretch (PO_2). This finding indicated that there was ionic crosslinking between nano-chitosan and TPP (linkage between phosphoric and ammonium ions).

Bulking and Retention

Mass and volume gain

The results of mass and volume gain of wood samples treated with vacuum impregnation process in both quaternized and non-quaternized nano-chitosan-TPP particles showed that quaternized nano-chitosan-TPP particles had a higher mass gain compared to control and non-quaternized nano-chitosan-TPP particles. Additionally, treatments 10, 14, and 16 increased mass gain in comparison to treatment 15 (Fig. 7). Treatments 10, 11, 13, 14, 16, and 17 resulted in a higher volume gain in comparison to treatments 12, 15, control, and non-quaternized nano-chitosan-TPP particles. Additionally, treatment 15 increased volume gain when compared to treatments 1, 4, 6, 8, and control (Fig. 8).

The contrast analysis revealed that mass gain and volume gain increased in quaternized nano-chitosan-TPP particles in comparison to non-quaternized nano-chitosan-TPP particles (Table 3). Additionally, quaternized nano-chitosan-TPP particles increased mass and volume gain relative to control (Table 3). Furthermore, crosslinking of TPP to nano-chitosan resulted in increased mass gain, but not in volume gain, in comparison to nano-chitosan particles without TPP. However, crosslinking of TPP to nano-chitosan particles exhibited higher volume gain compared to control (Table 3).

Mass gain can be expressed as weight percent gain (WPG), which is the increase in mass of wood specimen after treatment with preservatives or retention value. In fact, WPG is used to measure the efficacy of impregnation of preservatives in wood after treatment. Volume gain refers to bulking. According to Rowell (2012), cell wall bulking means that the wood is filled with the chemical when it reaches its green volume. The increased value of both mass and volume after treatment indicated the success of impregnation of nano-chitosan particles into the wood cell walls.

The mass and volume gain results indicated that both quaternized chitosan oligomers and quaternized-chitosan-TPP nanoparticles treated samples had more mass and volume gain after treatment in comparison with non-quaternized treated samples. Nowrouzi *et al*. (2015) used chitosan HMW (MW: 100 kDa to 300 kDa) to treat fir wood (*Abies sp*.) *via* impregnation and then heated wood samples at three different temperatures (60 °C, 80 °C, and 100 °C). It has also been reported that chitosan can only increase bulking to a small degree (less than 4%) and different temperatures did not influence penetration of chitosan into wood samples (Nowrouzi *et al*. 2015). In the current research, both quaternized and non-quaternized nano-chitosan-treated samples underwent changes in volume and mass that ranged from 4.9% to 18%. The LMW chitosan (MW: 50 kDa to 190 kDa) at 1/5% (v/v) concentration impregnated into radiata pine (*Pinus radiata*) (Singh *et al*. 2010). The results of light and SEM demonstrated that chitosan penetrated the cell lumens, small cavities, and cell walls (Singh *et al*. 2010). Therefore, dimensions of quaternized nano-chitosan particles seemed to be small enough to spread throughout wood elements and penetrate through pits and pores.

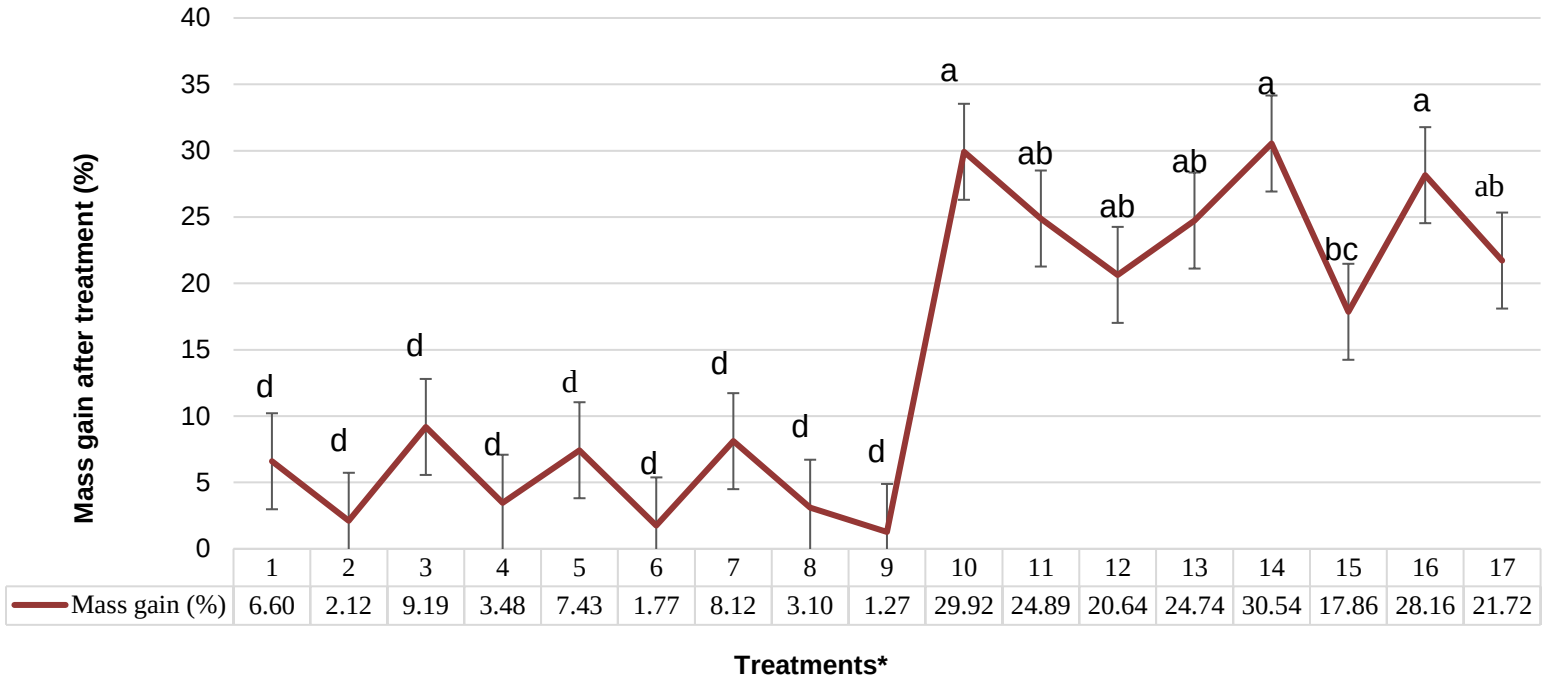

Fig. 7. Percentage of mass gain of wood samples after treating with vacuum impregnation process.
* All 17 treatments are described in Table 2.
[a-d] Means with no common superscripts differ significantly at p = 0.05 level

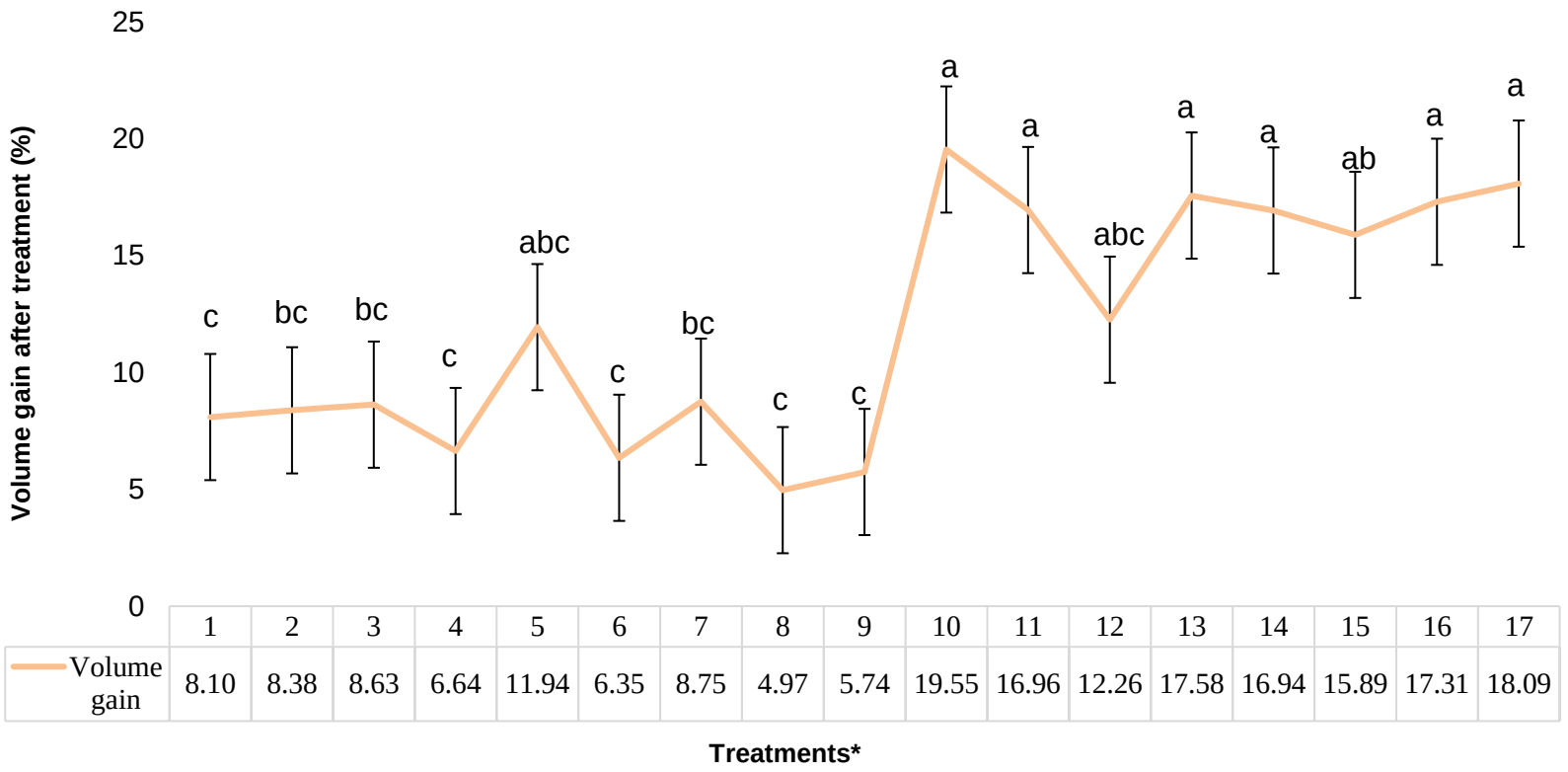

Fig. 8. Percentage of volume gain of wood samples after treating with vacuum impregnation process.
* All 17 treatments are described in Table 2.
[a-c] Means with no common superscripts differ significantly at p = 0.05 level

Table 3. Contrast Analysis for Mass Gain, Volume Gain, and Mass Loss

Contrasts	Mass Gain	Volume Gain	Mass Loss
Quaternized nano-chitosan-TPP *vs.* non-quaternized nano-chitosan-TPP	**	**	**
Quaternized nano-chitosan-TPP *vs.* control	ns	*	ns
Non-quaternized nano-chitosan-TPP *vs.* control	**	**	**
Nano-chitosan with TPP *vs.* nano-chitosan without TPP	**	**	ns
Nano-chitosan with TPP *vs.* control	**	**	*

ns = not significant.
*Treatment means within the same column within effect with no common superscripts are significantly different (P ≤ 0.05).
**Treatment means within the same column within effect with no common superscripts are significantly different (P ≤ 0.001)

Leaching

Mass loss

The results of mass loss after leaching of wood samples treated with vacuum impregnation process in both quaternized and non-quaternized nano-chitosan-TPP particles showed that quaternized nano-chitosan-TPP particles had higher mass loss compared to control and non-quaternized nano-chitosan-TPP particles. Additionally, non-quaternized nano-chitosan without TPP had increased mass loss compared to control (Fig. 9). The contrast analysis revealed that mass loss increased in quaternized nano-chitosan-TPP particles compared to non-quaternized nano-chitosan-TPP particles. Additionally, crosslinking of TPP to nano-chitosan resulted in increased mass loss (Table 3).

These results indicated that quaternized nano-chitosan particles penetrated the wood but could not fix into cell walls. In contrast, non-quaternized nano-chitosan particles remained in the wood cell walls. Generally, the formation of a strong covalent bond between chitosan and wood had not been previously reported. However, covalent bonds have been found between laccase-oxidized phenols in flax fibers and chitosan (Silva *et al*. 2011) and between laccase-oxidized phenolic acids and amino groups of chitosan (Aljawish *et al*. 2012). Fixation of chitosan by acetic anhydride has been reported by Mehrtens (1999). In the cited work the author reported that acetylated chitosan had lower mass loss after leaching, but the possible reason for fixation of chitosan was not described. Potentially, the fixation could be due to acetylation of wood. Treu *et al*. (2009) used copper in combination with chitosan to make a stronger fixation of chitosan into wood. Post-treatment at a temperature of 85 °C was used after impregnation. There, the author reported that post-treatment of chitosan-treated wood samples reduced the leaching of glucosamine, which is the main component of chitosan. Additionally, Nowrouzi *et al*. (2015) investigated the use of PEG (polyethylene glycol) along with chitosan to improve fixation and retention in fir wood specimens. A higher reaction occurred when PEG and heat were applied during the treatment process (Nowrouzi *et al*. 2015). That information may explain why in this study, although quaternized nano-chitosan particles were positively charged, they could not fix to cell walls and leached out.

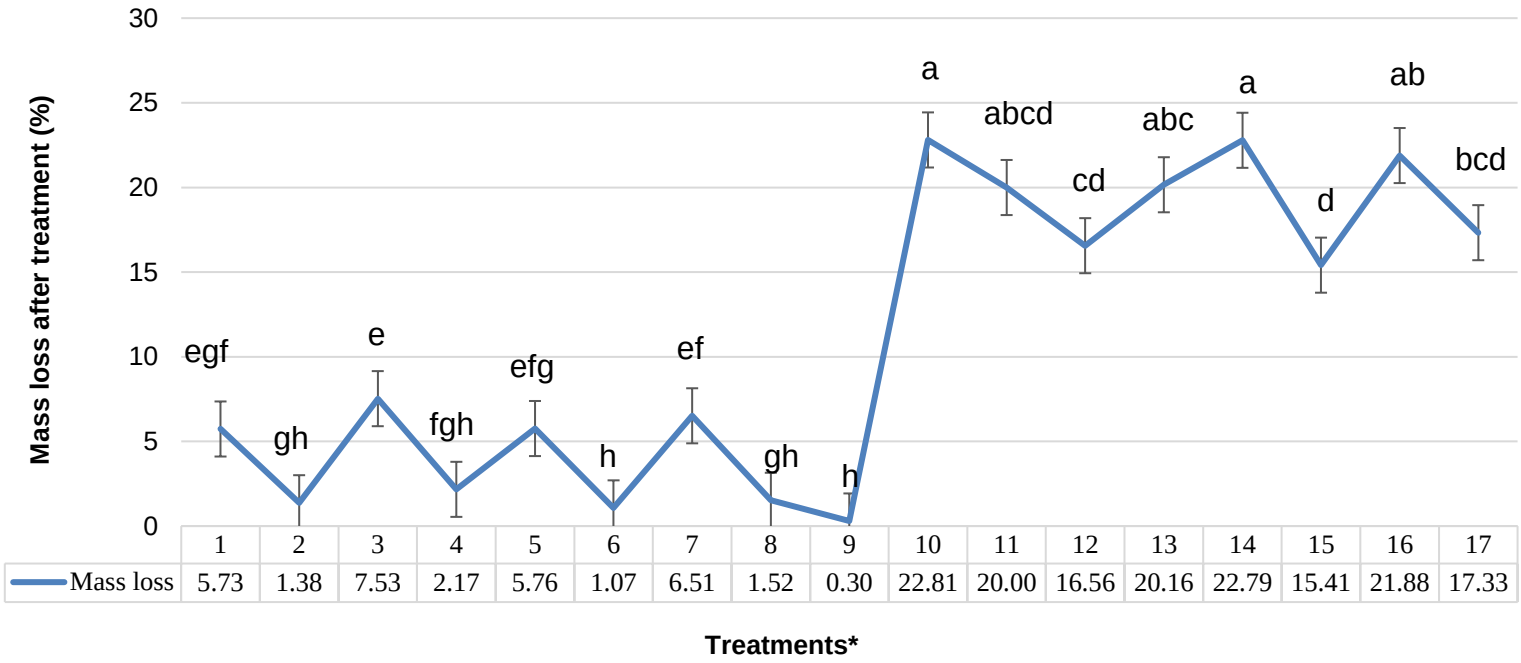

Fig. 9. Percentage of mass loss of leached wood samples
* All 17 treatments are described in Table 2.
[a-h] Means with no common superscripts differ significantly at p = 0.05 level

CONCLUSIONS

1. Compression strength results showed that hydroquinone (HQ) was the best mediator to be used with laccase for enzymatic modification of wood to increase the reactivity of wood and promote binding of nano-chitosan-TPP particles to wood.
2. Degree of polymerization of four with an applicable amount of sodium nitrite through depolymerization was successfully obtained and the size of chitosan oligomers was confirmed by thin layer chromatography.
3. The methylation of chitosan oligomers through the quaternization process and the formation of nano-chitosan-TPP particles were confirmed by FTIR spectra.
4. The result of bulking and leaching indicated that chitosan nanoparticles effectiveness was obtained in the short time period. It was essential that nano-chitosan particles penetrate and fix into the wood cell walls as well as cell lumens and cavities for long-term protection. These nanoparticles could potentially be used as wood preservatives to treat wood for interior applications. Further research is required to determine the exterior applications of nano-chitosan-TPP particles.

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