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Preparation of N-vanillyl chitosan and 4-hydroxybenzyl chitosan and their physico-mechanical, optical, barrier, and antimicrobial properties

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ABSTRACT

Chitosan derivatives such as N-vanillyl chitosan and 4-hydroxybenzyl chitosan were prepared by reacting chitosan with 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-hydroxybenzaldehyde. Amino groups on chitosan reacts with these aldehydes to form a Schiff base intermediate, which is later on converted into N-alkyl chitosans by reduction with sodium cyanoborohydride. The chemical reaction was monitored by ^{1}H NMR spectroscopy and the absence of aldehydic proton at 9.83 ppm in NMR spectra was observed for both the modified chitosan derivatives confirming the reaction. Modified chitosan films were later prepared by solution casting method and their physico-mechanical, barrier, optical and thermal properties were studied. The results clearly indicated significant change in tensile strength, water vapour transmission rate, and haze properties of modified chitosans. Modified chitosan films were also studied for their antimicrobial activity against *Aspergillus flavus*. The results showed a marked reduction of aflatoxins produced by the fungus in the presence of the N-vanillyl chitosan and 4-hydroxybenzyl chitosan film discs to 98.9% and non-detectable levels, respectively.

1. Introduction

Antimicrobial packaging is one of the most promising active packaging systems that have been found highly effective in inhibiting spoilage by killing pathogenic microorganisms that contaminate food. In this context, chitosan films have shown a great promise in their application in food preservation (Dutta, Shipra, Mehrotra, & Joydeep; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Salleh, Muhamad, & Khairuddin, 2007). Among various bioactive properties of chitosan, its antifungal activity has received considerable interest due to the problems associated with the fungicidal agents. Ghaouth, Arul, Asselin, and Benhamou (1992a,b), have reported that chitosan could inhibit the growth of *Alternaria alternate*, *Botrytis cinerea*, *Colletrotichum gloeosporioides*, and *Rhizopus stolonifer* and that the inhibitory index was affected by the concentration of chitosan. The growth of fungi such as *Fusarium oxysporium*, *R. stolonifer*, *Penicillium digitatum*, and *C. gloeosporioides* can be inhibited completely by chitosan at a concentration of 3% (Banos, Lopez, Molina, & Wilson, 2003; Banos et al., 2006). With regard to the antifungal activity of chitosan, researchers have focused on the effects of molecular weight and the degree of deacetylation. The effect of molecular weight on some antibacterial and anti-fungal activities have been explored by Chen (1998); Guo et al. (2007); Jia, Shen, and Xu (2001); Simojon and Fukushima (1996); Tokura, Miuray, Johmen, Nishi, and Nishimura (1994) synthesized Schiff bases of chitosan, N-substituted chitosan, and quaternized chitosan and measured their antifungal activities against *B. cinerea* Pers. (*B. cinerea* Pers) and *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium* (Pass) Ell.et halst). In addition Guo et al. (2006), prepared three kinds of Schiff bases of carboxymethyl chitosan (CMCTS) and analyzed their antifungal activities against *Valsa mali* (*V. mali*), *Alternaria solani* (*A. solani*), and *F. oxysporium* f. sp. *vasinfectum* (*F. oxysporium* f. sp. *vasinfectum*).

In this study, we report the synthesis of chitosan derivatives such as N-vanillyl chitosan and 4-hydroxybenzyl chitosan, and preparation, characterization, physico-mechanical, barrier and antimicrobial properties of the films.

2. Experimental

2.1. Materials

Chitosan powder from prawn shell (batch number BX 12AN-24) with a degree of deacetylation of 88% and viscosity of 195 mPas was obtained from India Sea Food, Cochin, India. The viscosity

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of chitosan was further confirmed in laboratory by using Rheology international RI: 3: M viscometer at an ambient temperature of (25 ± 2 °C). Vanillin (4-hydroxy-3-methoxybenzaldehyde) and 4-hydroxybenzaldehyde of analytical grade was purchased from Sisco Chemicals, Mumbai, India and it was used without further purification. Sodium cyanoborohydride was procured from Sigma Aldrich, St. Louis, MO, USA. Aflatoxin standards B_1, B_2, G_1 and G_2 were purchased from Sigma–Aldrich Co. All other chemicals were of analytical grade obtained from M/s S D fine Chemicals, Bangalore, Karnataka (India).

2.2. Reaction of chitosan with aldehydes [4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-hydroxybenzaldehyde]

Chitosan (1.00 g, 6.11 mmol of D-glucosamine repeat unit) was dissolved in 70 ml of 0.2 M acetic acid (pH 4). The solution was later diluted with 70 ml of ethanol, and then an aldehyde (1 g equivalent of GlcN) was added to the solution. The reaction mixture was stirred for 1 h. At this point the pH of the solution was adjusted to 5 by adding 1 M sodium hydroxide solution followed by the addition of sodium cyanoborohydride (1.53 g, 24.46 mmol) to the resulting solution. The solution was allowed to stir at room temperature for 24 h, followed by adjusting the pH to 7 with 15% (w/v) sodium hydroxide. The precipitated was washed with ethanol and water several times followed by acetone wash and dried at room temperature under nitrogen atmosphere (Muzzarelli & Ilari, 1994; Muzzarelli, Ilari, Xia, Pinotti, & Tomasetti, 1994; Warayuth et al., 2006).

2.3. Preparation of plain chitosan and chemically modified chitosan films

2% solutions of both plain chitosan and chemically modified chitosans were prepared by dissolving 2 g of each plain chitosan and modified chitosan in 100 ml of 1% acetic acid with constant stirring. The viscous solutions prepared were later filtered through a cheese cloth to remove any impurities. Later, both the solutions were degassed using vacuum pump to remove entrapped air. Approximately 200 ml of the above mentioned solutions prepared were filtered through cotton and poured on separate glass plates (27 cm × 20 cm) lined with a polyethylene film. The solutions in the glass plates were allowed to dry at room temperature (3–4 days) to obtain a transparent film with uniform thickness. After drying, the films were peeled off and stored at ambient conditions (25 °C, RH of 50%).

2.4. ^1H NMR spectroscopy

Five milligrams each of plain chitosan and chemically modified chitosan were dissolved in 600 μl D_2O with 2% deuteriated trifluroacetic acid. ^1H NMR was recorded at 500 MHz on Bruker Avance NMR spectrometer (Rheinstetten-Germany) with deuterium lock signal tuned at 76.753 MHz.

2.5. Physico-mechanical properties

The physico-mechanical properties like tensile, elongation at break, tear strength and burst strength for plain and chemically modified chitosan films were evaluated after equilibrating at 65% RH at 27 °C.

The tensile strength (TS) and elongation at break of plain chitosan and chemically modified chitosan films were measured as per ASTM D – 882 method, using LLOYD's Universal Testing (LLOYDS-50KN London U.K.) instrument. Tear strength of plain chitosan and chemically modified chitosan films were measured using an Elmendorf tear tester (H.E. Messmer, London) as per ASTM D 1922. Burst strength of films was measured using a Linux burst tester (model no. 550, Linux machines incorporation, Thane, India) as per ASTM D 774. The average of five measurements with standard deviation of above mentioned properties are reported.

2.6. Barrier properties

Water vapour transmission rate WVTR (Chinnan & Park, 1995) of plain chitosan and chemically modified chitosan films were determined using aluminum dishes as per ASTM E-96-95. The average of four measurements is reported with standard deviation.

2.7. Optical properties

The optical properties of plain chitosan and chemically modified chitosan films were measured using a Suga test, Digital Hazemeter (model HGM-2DP Japan) after equilibrating the samples at 65% RH at 27 °C. The haze behaviour of dust and grease free films was recorded as per ASTM D-1003-61 method.

2.8. Thermal properties

2.8.1. Differential scanning calorimetry

Studies on various melting and crystallization parameters of plain and chemically modified chitosan films were determined by differential scanning calorimeter (Q-200, TA Instruments, Delaware, USA). Temperature and heat flow calibration of the equipment was maintained with standard indium (99%) under conditions similar to those employed in the experiments with the samples. All the experiments were carried out with sealed empty pan as the reference, with N_2 gas flushing. Sealed pans with samples (5–10 mg) were first cooled to −50 °C, held isothermally for 1 min, and then ramped (20 °C/min) to 250 °C to obtain heat flow curves. Temperature onset, crystalline melt temperature (Tp), temperature of completion of the endotherm during melting, exotherm during crystallization and heat of enthalpy (ΔH) were obtained on thermograms using TA universal thermal analyzer software (Universal V4.3A, TA instruments).

2.8.2. Thermo-gravimetric analysis (TGA)

A thermal weight change analysis instrument (TGA, Q50, TA Instruments, Delaware, USA), was used to measure the amount and rate of change in weight of the material as a function of increasing temperature or time, in a controlled atmosphere. The plain chitosan and chemically modified chitosan film samples (8–10 mg) were kept in a platinum crucible and heated in the furnace, from 0 to 700 °C, at the rate of 20 °C/min, and nitrogen stream was out flushed at the rate of 40 ml/min. The percentage of weight loss was plotted against temperature.

2.9. Statistical analysis

The significant differences between means of samples were determined by '*t*'-test using Microsoft Excel. Significance of differences was defined at $p \leq 0.05$.

2.10. Antimicrobial activity of modified chitosan films

Fungal strain *Aspergillus flavus* MTCC 2798 examined in the study was procured from (MTCC) Microbial Type Culture Collection (http://mtcc.imtech.res.in/catalogue.php), Institute of Microbial Technology, Sector39-A, Chandigarh, India.

2.10.1. Studies on antifungal activity of prepared films by direct exposure method

The fungus used in the study was *A. flavus*, an aflatoxigenic strain, capable of producing aflatoxin B_1 and B_2 on synthetic media. 50 ml of Czapek dox broth was taken in 250 ml conical flasks. Twenty discs each of plain chitosan and chemically modified chitosan films were suspended in the media and the flasks were sterilized by autoclaving. Fungal spore suspension of 13,000/ml was prepared by direct counting under the microscope, and 1 ml was added to each flask. All treatments were in triplicates. The flasks were incubated in dark for 13 days as stationary cultures. After the period of incubation, 50 ml of methanol was added to each flask and shaken well. The contents of each flask were passed through preweighed Whatman 4 filter paper circles to collect the biomass. The filter paper circles were dried and weighed to determine the fungal biomass. The filtrate was taken in a separating funnel and extracted three times with chloroform (20 ml + 20 ml + 10 ml) and the resultant chloroform layer was passed through a sodium sulphate bed and the collected extract was evaporated to dryness. The contents were resuspended in 1 ml methanol, stirred to dissolve the toxin, and diluted with 1 ml milli Q water. Aflatoxin concentration was determined in these extracts by liquid chromatography.

2.10.2. Aflatoxin determination by reversed phase liquid chromatography

LC system was Model Hewlett Packard (HP 1100 series module with HP Chemstation for instrument control, data acquisition and data evaluation) with fluorescence detector. LC column: Waters, 4.6 mm × 25 mm Symmetry C 18 column (Waters Corporation 34 Maples Street, Milford, MA, USA). Operating conditions: Flow rate 1 ml/min, excitation and emission wavelengths were 360 nm and 440 nm, respectively. The UVE photochemical reactor (LC Tech GmbH, Germany) placed between the LC column and the detector was used for the post-column photochemical derivatization of aflatoxin B_1 and aflatoxin G_1. Mobile phase was water:methanol:acetonitrile (62:22:16), Isocratic mode and injection volume was 50 μl through a standard loop.

Aflatoxin standard stock solutions approximately 10 μg/ml were prepared in benzene–acetonitrile (98 + 2). Concentrations of individual stock solutions were determined by measuring UV absorbance. Actual concentration of the individual aflatoxin solution was determined using the equation below. The molecular weights and molar absorptivities (ε) in benzene:acetonitrile (98 + 2) of aflatoxin B_1, B_2, G_1 and G_2 were 312, 314, 328 and 330 and 19,800, 20,900, 17,100 and 18,200, respectively.

$$\text{Aflatoxin } (\mu g/ml) = \frac{\text{Absorbance} \times \text{molecular weight} \times \text{correction factor}}{\text{Molar absorptivity}(\varepsilon)}$$

Appropriate aliquots of the four aflatoxins were transferred into four vials as mixtures of the four toxins at different concentrations. Solvent was evaporated to dryness and the toxins dissolved in 1 + 1 ml of methanol and water. Fifty microliter volumes were injected into the HPLC system and responses recorded. To prepare standard curves of the four aflatoxins, the peak area was plotted against the concentration of the toxins and the calibration curve calculated using linear regression. The calibration curves of the four aflatoxins exhibited linearity with correlation greater than 0.995. The limit of detection (LOD) was 0.3 ng ml^{-1} of AFB_1 and AFG_1, and 0.15 ng ml^{-1} of AFB_2 and AFG_2 on a signal to noise ratio of >3.

3. Results and discussion

3.1. Reaction mechanism

The mechanism envisaged for the reaction of chitosan with 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-hydroxybenzaldehyde is shown in Fig. 1. Amino groups on chitosan reacts with these aldehydes to form a Schiff base intermediate, which is converted into N-alkyl chitosans. The reduction of aldimine was performed using $NaCNBH_3$, which is more reactive and selective than other usual reducing agents such as sodium borohydride ($NaBH_4$), selephenol (PhSeH) or pentacarbonyl ion in alcoholic potassium hydroxide. The advantage of such reducing agent is its stability in the acidic media, its hydrolysis rate at pH 3 being smaller than other common reducing agents and at a pH of 7 it is only 0.5 mol% after 24 h. Moreover the reduction of aldimine by BH_3CN^- anion is rapid at pH values of 6–7 and the reduction of aldehydes or ketones is negligible in this pH range (it becomes quick at pH values smaller than 3.5). This is in accordance with earlier studies (Desbrieres, Martinez, & Rinaudo, 1996; Guo et al., 2006, 2007; Jia et al., 2001; Muzzarelli & Ilari, 1994; Muzzarelli et al., 1994; Warayuth et al., 2006).

3.2. Chemical characterization by ^1H NMR spectroscopy

^1H NMR spectra of chitosan, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxybenzaldehyde and

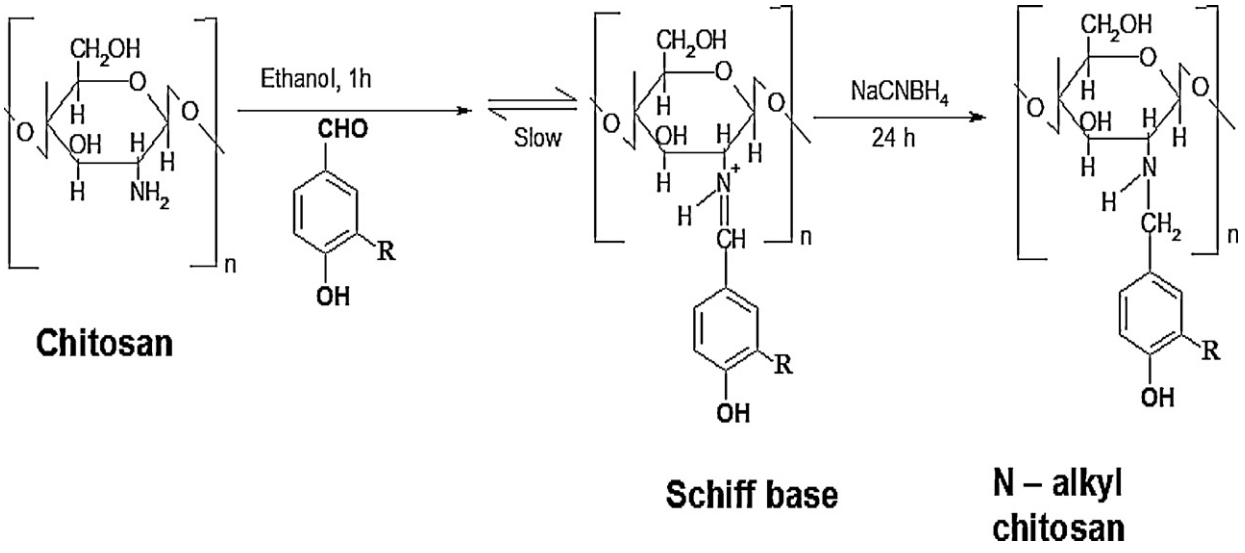

Fig. 1. Chemical modification of chitosan with vanillin (R-OCH_3) and 4-hydroxybenzaldehyde (R-H).

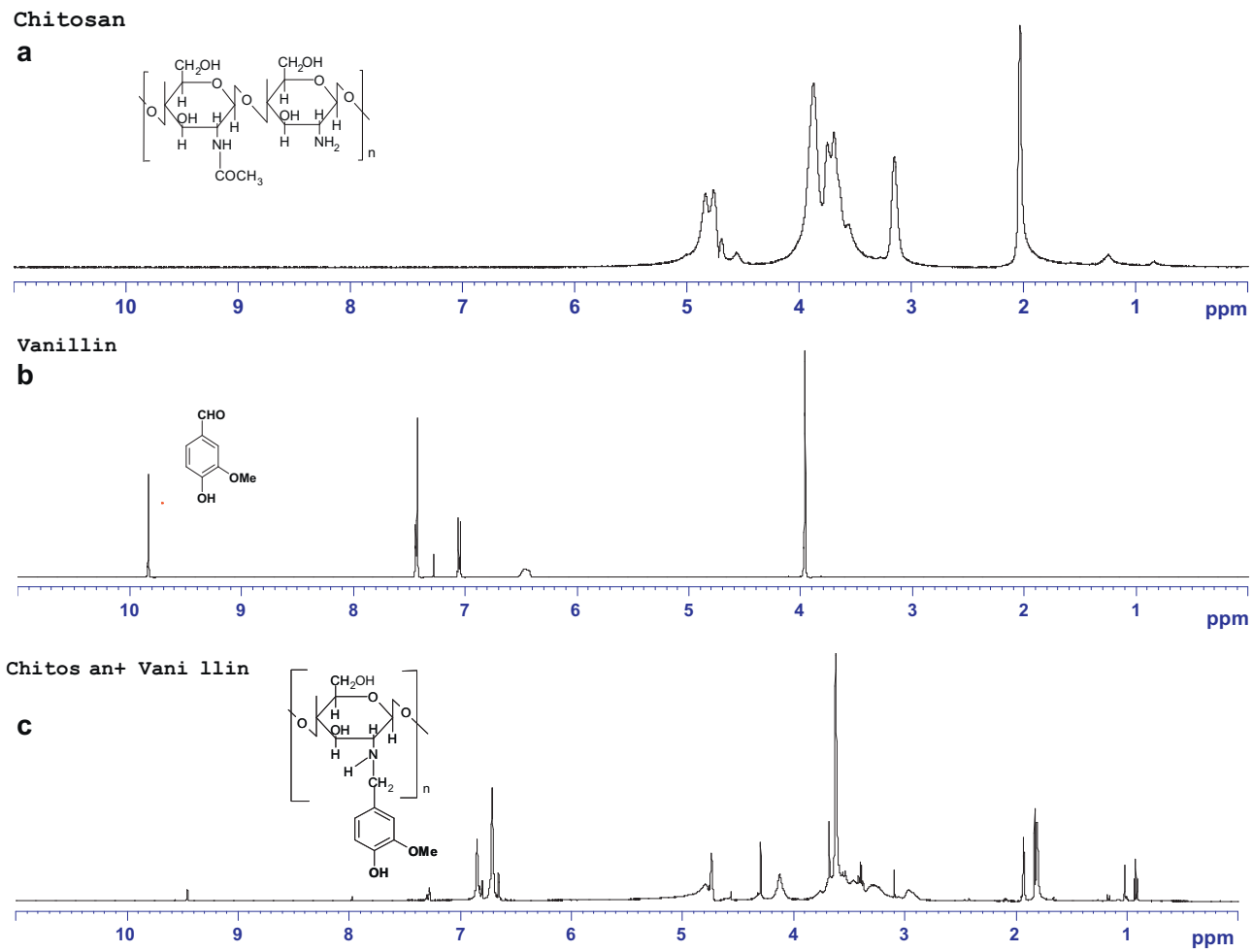

Fig. 2. ^{1}H NMR characteristics of (a) chitosan, (b) vanillin and (c) chemically modified chitosan with vanillin (N-vanillyl chitosan).

corresponding chemically modified N-alkyl chitosans are presented in Figs. 2 and 3, respectively. Figs. 2a and 3a show the ^{1}H NMR spectrum of chitosan with anomeric proton (H1) as a multiplet at 4.80 ppm along with multiplets at 3.40–4.20 for H-3, H-4, H-5, H-6 and H-6′ and 3.15 ppm for H-2 proton. The signal at 2.03 ppm corresponds to the residual acetyl protons in the deacetylated chitosan material. In Fig. 2b, the ^{1}H NMR spectrum of vanillin shows aldehydic proton at 9.83 ppm, aromatic protons as multiplets at 7.44 (2H) and 7.05 ppm (1H), phenolic proton as a broad singlet at 6.48 ppm and the methoxyl protons at 3.96 ppm. In the ^{1}H NMR spectrum of vanillyl chitosan (Fig. 2c) shows the absence of aldehydic proton indicating the reaction occurring at this position/group. Earlier studies reported formation of Schiff base in reaction of chitosan with other aldehydes followed by reduction for the synthesis of N-alkyl chitosan derivatives (Desbrieres et al., 1996; Guo et al., 2006, 2007; Jia et al., 2001; Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982; Rinaudo, Desbrieres, Dung, & Dong, 2001; Warayuth et al., 2006). Also the ratio of intensity of the anomeric proton at 4.29 ppm to that of aromatic protons at 6.80 ppm clearly demonstrated that the degree of substitution was around 63%. Similarly, Fig. 3c depicting ^{1}H NMR spectrum for 4-hydroxybenzyl chitosan derivative exhibits representative aromatic protons for the alkyl position at 7.49 and 7.09 ppm along with the protons for chitosan and benzyl protons at 5.15, 4.73, 3.6–4.2, 3.52 and 2.19 ppm. The degree of N-alkyl substitution as determined by ratio intensity of anomeric proton at 5.15 and aromatic protons at 7.49 ppm is around 59%.

3.3. Physico-mechanical properties

Physico-mechanical properties of chitosan and chemically modified chitosan films with vanillin and 4-hydroxybenzaldehyde were compared with plain chitosan in Table 1. It was observed that N-vanillyl chitosan and 4-hydroxybenzyl chitosan films showed an increase in tensile strength up to 17.7% and 11.11%, while a decrease in percentage elongation at break 30% and 17%, tear strength lowered by 37% and 25% and burst strength decreased by 24.59% and 14.75%, respectively. The increase in tensile strength of modified films could be due to the covalent bonding of chitosan with vanillin and 4-hydroxybenzaldehyde, the decrease in % elongation, tear and burst strengths may be due to the brittleness of films.

3.4. Barrier properties

Water vapour transmission rate (WVTR) of native chitosan films was 4410.73 g/m^2/days. Chemical modification of chitosan with vanillin and 4-hydroxybenzaldehyde affects the WVTR of chitosan films significantly and is shown in Table 1. WVTR of chemically modified chitosan films decreased to 68% in N-vanillyl chitosan and 79% in 4-hydroxybenzyl chitosan derivatives, respectively. This decrease in WVTR of modified chitosan films could be due to the chemical modification of chitosan with vanillin as well as chitosan and 4-hydroxybenzaldehyde, which might reduce the polarity of the derivative thereby influencing water retardation.

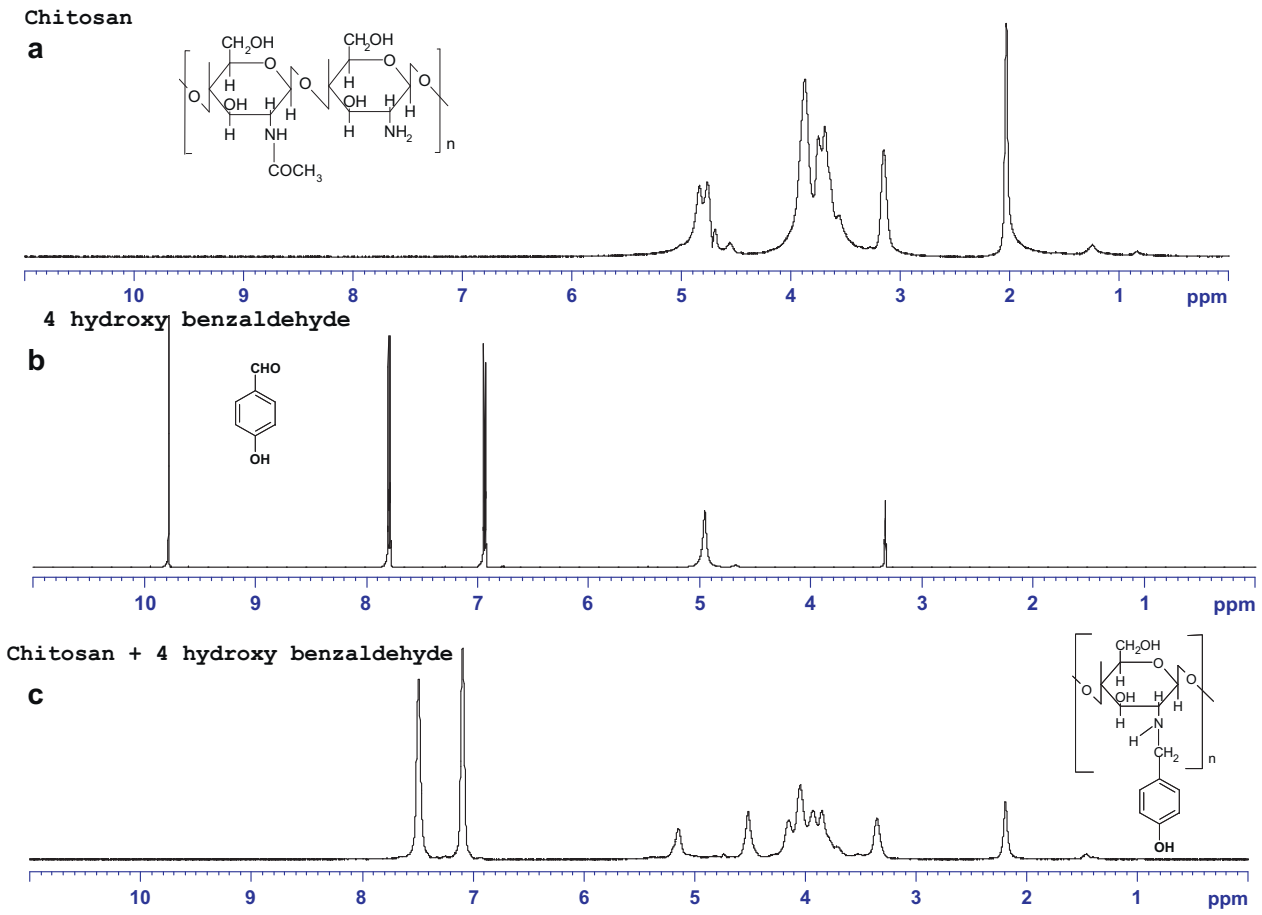

Fig. 3. ^{1}H NMR characteristics of (a) chitosan, (b) 4-hydroxybenzaldehyde and (c) chemically modified chitosan with 4-hydroxybenzaldehyde (4-hydroxy benzyl chitosan).

3.5. Optical properties

Haze values studied for plain chitosan and chemically modified chitosan films are shown in Table 1. Haze values decreased marginally from 11 in plain chitosan to 10.3 for N-vanillyl chitosan and to 9.9 for 4-hydroxybenzyl chitosan films indicating the improvement in their transparencies. This may be due to the decrease in crystallinity of the modified chitosan derivatives.

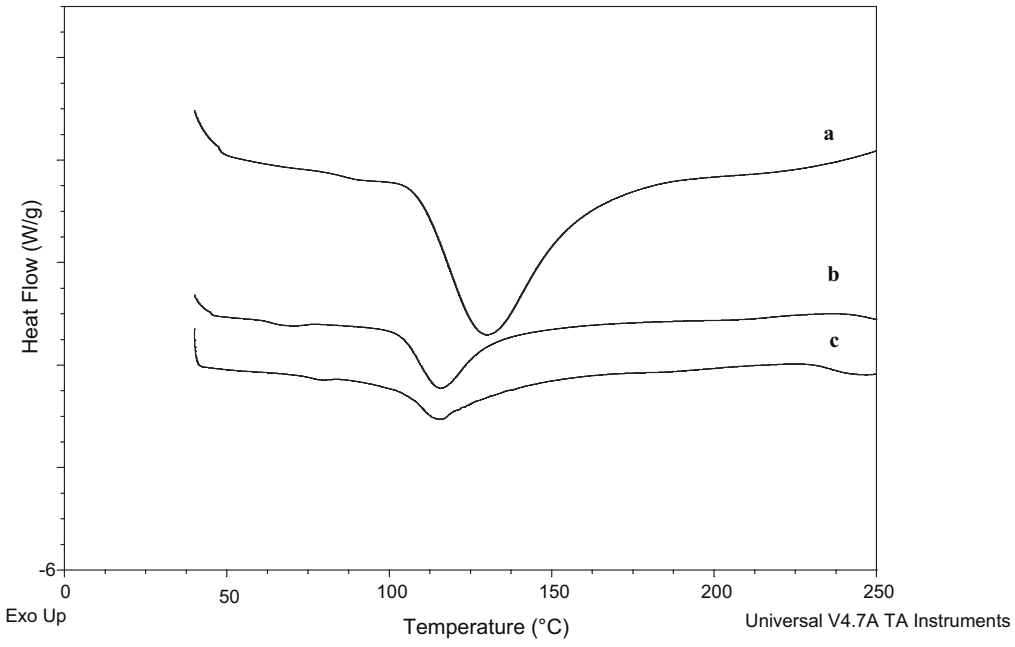

Fig. 4. DSC thermograms (a) chitosan, (b) N-vanillyl chitosan and (c) 4-hydroxy benzyl chitosan films.

Table 1
Physico-mechanical, WVTR and optical properties of chitosan and chemically modified chitosans films.

Materials films	Tensile strength (MPa)	% Elongation	Burst strength (Kg/cm^2)	Tear strength (g)	WVTR g/m^2/days at 90% RH gradient at 38 °C	Haze
Plain chitosan	45 ± 0.79^a	16.31 ± 0.92^a	6.1 ± 0.42^a	12 ± 0.56 ^{a}	4410.73 ± 63.28^a	11.0 ± 0.12^a
N-vanillyl chitosan	53 ± 0.54^b	11.34 ± 0.39^b	4.6 ± 0.31^b	7.5 ± 0.68^b	1396.80 ± 27.01^b	10.3 ± 0.19^b
4-Hydroxy benzyl chitosan	50 ± 0.47^c	13.52 ± 0.48^c	5.2 ± 0.57^c	9.0 ± 0.83^c	900.00 ± 15.82^c	9.9 ± 0.07^c

The data with different superscripts (a, b and c) differ significantly at the probability level $p \leq 0.05$.

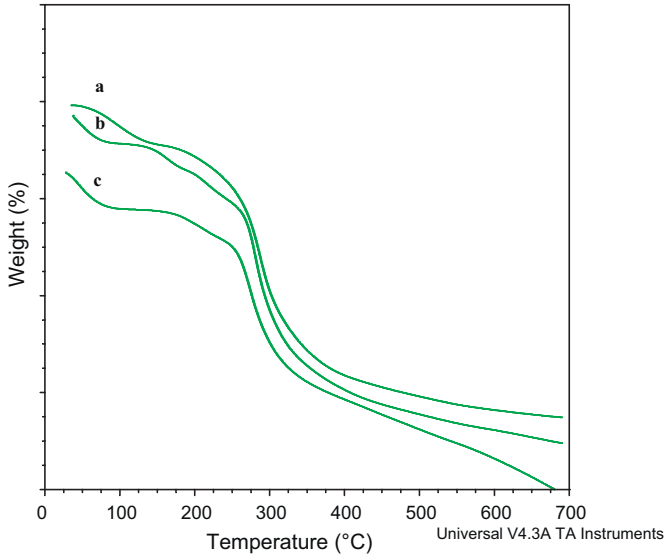

Fig. 5. TGA curves of (a) chitosan, (b) N-vanillyl chitosan and (c) 4-hydroxybenzyl chitosan films.

3.6. Thermal properties

The typical DSC thermograms for the heating curves of the plain chitosan and modified chitosans are shown in Fig. 4. The values of endothermic peak (Tp) decreased from 129.36 °C for chitosan to 115.83 °C and 114 °C for both N-vanillyl chitosan and 4-hydroxybenzyl chitosan systems, respectively. This decrease in the values of endothermic peak (Tp) is attributed to evaporation of residual water. The enthalpy value 299.6 of chitosan decreased to 144.5 J/g for vanillin based chitosan and 120.2 J/g for 4-hydroxybenzaldehyde based chitosan due to the chemical modification (Mucha & Pawlak, 2005; Yang, Dou, Liang, & Shen, 2005).

The typical thermograms of chitosan, N-vanillyl chitosan and 4-hydroxybenzyl chitosan are shown in Fig. 5 TGA of chitosan and its derivatives shows gradual percent weight loss up to 10–15% up to 200 °C due to the loss of adsorbed and bound water dehydration. There was a sudden percent loss in weight from 35 to 42% at 300 °C due to the decrosslinking of polymer network and finally the percent weight loss falls to 94% in chitosan and 66–67% in both the derivatives at 700 °C. The results indicate a better thermal stability with modified chitosan films (Simi & Abraham, 2010).

3.7. Antifungal activity of prepared films

In the study with the fungal culture *A. flavus* both the biomass and aflatoxin production was determined. The fungal strain used in the study produced only aflatoxin B_1 and B_2 as indicated by the HPLC analysis (Fig. 6). In the study it was observed that the reduction in biomass was only 17 and 13% in the N-vanillyl chitosan and 4-hydroxybenzyl chitosan films, respectively when compared to the plain chitosan films (Table 2). However, a marked reduction of aflatoxins produced by the fungus in the presence of the modified chitosan discs was observed when compared with the plain chitosan discs. In the case of vanillin modified film aflatoxin B_1

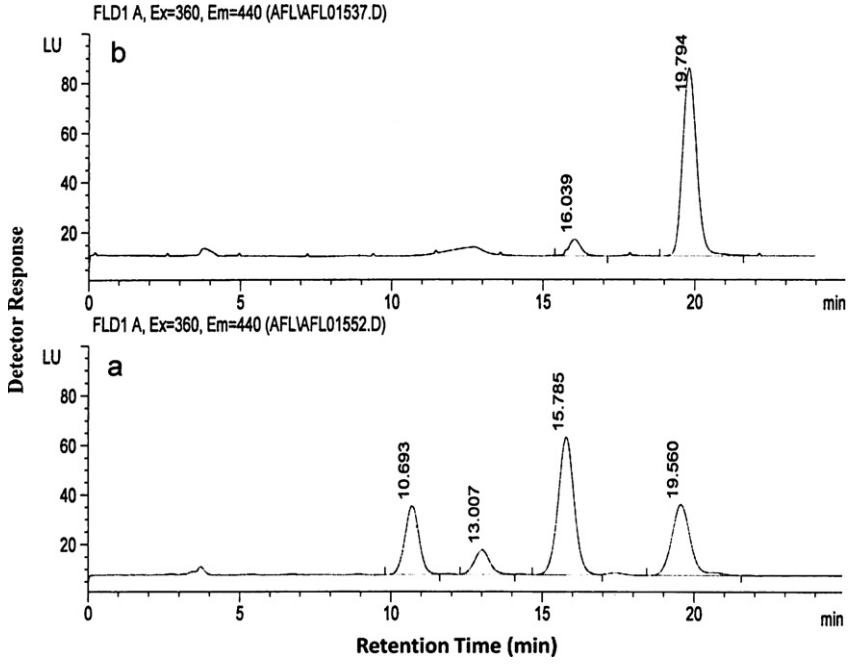

Fig. 6. (a) Chromatograms of standard aflatoxins G_2, G_1, B_2 and B_1 and (b) chromatograms aflatoxin B_2 and B_1 by *A. flavus* in synthetic media incorporated with discs of chitosan and chemically modified chitosan films.

Table 2
Production of aflatoxin B_1 and aflatoxin B_2 by *A. flavus* in synthetic media incorporated with discs of chitosan and chemically modified chitosan films.

Materials Film	Dry weight of fungal mass (g)	Aflatoxin produced (μg/ml of culture filtrate)	
		Aflatoxin B_1	Aflatoxin B_2
Plain chitosan	0.46 ± 0.014^a	6.93 ± 0.24^a	0.28 ± 0.029
N-vanillyl chitosan	0.38 ± 0.019^b	0.10 ± 0.01^b	ND
4-Hydroxy benzyl chitosan	0.40 ± 0.014^b	ND	ND

The data with different superscripts (a and b) differ significantly at the probability level $p \leq 0.05$. ND, not detected, $n = 3$.

production was reduced by 98.9% and aflatoxin B_2 was reduced to non-detectable levels. A more severe effect was observed with regard to the 4-hydroxybenzaldehyde modified films, since production of both aflatoxin B_1 and B_2 were reduced to non-detectable levels. This was particularly noteworthy as the calculated weight of the films of N-vanillyl chitosan and 4-hydroxybenzyl chitosan incorporated into the media was only 0.36 and 0.52 mg/ml (ppm). Antimicrobial activity of Schiff bases of N-substituted chitosan, carboxy methyl chitosan (CMCTS) and carboxybutyl chitosan were observed by Guo et al. (2006, 2007) and Muzzarelli et al. (1990). Chitosan has also been reported to inhibit spore germination, germ tube elongation and radial growth (Ghaouth et al., 1992a,b) but this is the first report of decreased or non production of a mycotoxin by a fungus when in contact with chitosan based films. Eweis, Elkholy, and Elsabee (2006), have observed that chitosan oligomers diffuse inside hyphae interfering with the enzymes responsible for the growth of the fungus. The intensity of degradation action on the fungal cell walls is also dependent upon the concentration, degree of acetylation and local pH.

4. Conclusions

Chitosan was effectively modified chemically with vanillin and 4-hydroxy benzaldehyde with a degree substitution of 63% and 59%, respectively of the amino group. It was observed that the water vapour transmission rate decreased by 68% and 79.59% and that of haze properties decreased by 6.3% and 10% in both chemically modified films with vanillin and 4-hydroxybenzaldehyde, respectively. Similarly, strength properties also showed a significant improvement in tensile strength up to 17.7% and 11.11% in N-vanillyl chitosan and 4-hydroxybenzyl chitosan derivatives, respectively. N-vanillyl chitosan and 4-hydroxybenzyl chitosan film discs showed a marked reduction of aflatoxins produced by the fungus to 98.9% and at non-detectable levels, respectively and ascertaining its compatibility for food packaging applications.

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