

ASIAN JOURNAL OF CHEMISTRY



https://doi.org/10.14233/ajchem.2021.22980

Synthesis and Characterization of Chitosan from Prawn Shells and Study of Its Effects on Weight Loss of *Myrica esculenta* Fruits

S. Pokhrel^{1,*,0}, S. Shah¹ and H.S. Adhikari²

¹Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

Received: 3 September 2020;

Accepted: 22 October 2020;

Published online: 15 January 2021;

AJC-20212

In this work, chitin and chitosan were obtained from prawn shell wastages by chemical treatment method. Structural characterization of chitin and chitosan by FTIR, X-ray photoelectron spectroscopy (XPS) and ¹³C NMR clearly showed the formation of chitosan from chitin. The physico-chemical properties of chitosan *viz*. molecular weight, moisture content, ash content and degree of deacetylation (DD) were analyzed. The optimum condition of deacetylation process to obtain chitosan from chitin was analyzed. The FTIR spectra showed the characteristic peaks corresponding to hydroxy, acetamido and amino functionalities of chitosan obtained from partial deacetylation of chitin and the solid state ¹³C NMR showed the formation of chitosan with characteristic peaks. XRD showed the shifting of crystallinity phases showing more crystallinity of chitin than chitosan. XPS spectrum of prepared chitin with the peaks corresponding to N, C and O binding energy was analogous to the standard. The effect of chitosan coating in extension of postharvest life of Kaphal (*Myrica esculenta*) fruits was investigated and chitosan coating was observed to have a potential to prolong storage life, control decay and weight loss.

Keywords: Chitosan, Chitin, Myrica esculenta, Postharvest shelf life, Weight loss.

INTRODUCTION

Chitin is the second most abundant material in nature after cellulose [1]. It is extracted from the exoskeletons and the internal structure of invertebrates such as crustaceans, insects, mollusks, honeybees, silkworms, etc. and it also constitutes in the structure of many fungi and yeast [1-3]. Unlike most other polysaccharides, chitin contains 5-8% nitrogen which makes it useful as a chelating agent [1]. Due to biocompatibility, biodegradability and non-toxicity, chitin is more useful in medicine, agriculture, cosmetic, food and wastewater treatment [4,5]. In native state, natural chitin exists in three forms i.e. α -, β - and γ -forms depending on the source [6]. Studies on chitin and chitosan have been increased since 1990 because of their excellent biological properties such as biodegradation in the human body, immunological [7], antibacterial [3,8] and wound-healing activity [2,9]. Chitin is chemically (1,4)-2amino-2-deoxy-β-D-glucan, a unique polysaccharide [10] and chitosan is a value-added biomaterial obtained by deacetylation of chitin [11]. Besides the traditional commercial fruits, the

wild fruits are also gaining increased attention as potential food supplement or cheaper alternative of commercial fruits all over the world [12].

Myrica esculenta (family Myricaceae), commonly known as Kaphal, an edible fruit used for other by-products as well, is highly valuable wild fruit with potential income-generating species in the sub-Himalayan region [13]. The fruits are succulent drupe with small ellipsoidal or ovoid to globose in shape, initially green and become reddish during ripening [14]. These fruits are highly perishable in nature and their shelf life does not exceed 2-3 days [15]. Chitin and chitosan are non-toxic biomaterials, so they are useful in food industry as flavouring and colouring agents and as dietary fibers in baked food [2,14]. Chitosan prolongs storage life and controls decay of fruits by reducing the growth of many phytopathogenic bacteria and fungi because of its semipermeable film forming ability and biochemical properties [16]. Therefore, the aim of present work was to study the effect of chitosan coating on weight loss of kaphal fruits (Myrica esculenta).

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

²Department of Applied Sciences, Western Region Campus, Institute of Engineering, Tribhuvan University, Pokhara, Nepal

^{*}Corresponding author: Tel: +977 9841409842; E-mail: shantabhattarai2014@gmail.com

EXPERIMENTAL

Prawn shells were collected from the local market of Kathmandu, Nepal. They were washed with water to remove the loose tissue and properly dried in sun. Potassium hydroxide (≥84% Merck), sodium hydroxide (97% Merck), hydrochloric acid (95% Fisher Scientific), ammonium acetate (98.5% Fisher Scientific) and acetic acid (99.5% Fisher Scientific) were of analytical grade and used without further purification.

Preparation of chitin and chitosan from prawn shells: The prawn shells were washed, dried and powdered with the help of grinder. The powder was filtered through 600 μm mesh sieves and subjected for demineralization and deproteinization to obtain chitin by following the established protocol [17] with some modifications. The effect of time variation and KOH concentration on the deacetylation process of chitin was studied. Such obtained chitin was deacetylated with KOH solution in a 1:10 (w/v) ratio [3] at 100 °C for different intervals of time (0.5, 1.0, 2.0, 3.0 and 4.0 h) and different concentrations of KOH (45, 55, 65 and 75%). The products obtained were washed until neutrality reached and the resultant chitosan was sun-dried for 7 days. The physico-chemical properties of the prepared samples are presented in Table-1.

Determination of moisture content: The moisture content in prepared chitosan was determined by the gravimetric method. In this process, the crucible was heated at 100 °C for 15 min, cooled in a desiccator and weighed. A chitosan sample (0.5 g) was taken in a weighed crucible, dried into oven at 100 °C for 3 h and the process was repeated till the constant weight was obtained [18]. The moisture content was determined using eqn. 1:

Moisture content (%) =
$$\frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100 (1)$$

Determination of ash content: Ash content of chitosan was determined by combustion using a constant weight crucible. Chitosan (2 g) was combusted in the constant weight crucible in an oven at 550 ± 20 °C for 3 h. The crucible was removed, cooled in a desiccator for 30 min and reweighed (W₁). This method of heating and cooling was repeated in every 1.5 h until a constant weight (W₂) was established [19]. The ash percentage was calculated by eqn. 2:

Ash (%) =
$$\frac{W_2 - W_0}{W_1 - W_0} \times 100$$
 (2)

where W_0 is the constant weight of crucible, W_1 is the weight of sample and crucible, W_2 is the weight of ash and crucible.

Determination of molecular weight: The molecular weight of chitosan was determined by viscosity-average molecular weight (Dalton) method. Chitosan was dissolved in a

mixture of 0.15 M ammonium acetate and 0.2 M acetic acid solution at 30 °C. The intrinsic viscosity (η) was determined with the help of Ostwald's viscometer [3] using Mark-Houwink equation (eqn. 3):

$$[\eta] = KM^a \tag{3}$$

Molecular weight (M) of chitosan was determined by relating intrinsic viscosity with empirical viscometric constants $K = 9.66 \times 10^{-5}$ dm³/g and a = 0.742 for chitosan.

Determination of degree of deacetylation

Acid base titration: The degree of deacetylation showed the percentage of *N*-acetyl groups replaced by amino groups (NH₂) from the chitin to produce chitosan. Degree of deacetylation was measured by the following protocol [3] with some modifications. Chitosan (0.3 g) was dissolved in 0.1 M HCl (30 mL) at room temperature with constant stirring and two drops of methyl orange indicator were added followed by titration with 0.1 M NaOH solution. The end point was indicated by the change in colour from pink to orange yellow. Three parallel samples were used. The percentage of free NH₂ groups in chitosan was calculated [3,20] as follows:

NH₂ (%) =
$$\frac{[(C_1V_1 - C_2V_2) \times 0.016]}{[G(100 - W)]} \times 100$$
 (4)

Free NH₂ (%) =
$$\frac{\text{NH}_2\%}{9.94\%} \times 100$$
 (5)

Theoretical NH₂ content (%) =
$$\left(\frac{16}{161}\right) \times 100 = 9.94\%$$
 [20]

where C_1 = concentration of HCl (M); V_1 = volume of HCl added (mL); C_2 = concentration of NaOH (M); V_2 = volume of NaOH added by titration (mL); G = sample weight (g); W = moisture content (%); 0.016 = NH₂content (g) in 1 mL of 1 M HCl [20].

FTIR method: The degree of deacetylation of prepared chitosan samples was determined by FT-IR spectroscopic method [21,22] using the eqn. 6:

DD (%) =
$$100 - \frac{1}{0.03133} \left(\frac{A_{1320}}{A_{1420}} - 0.3822 \right)$$
 (6)

where, A is absorbance, DD is the degree of deacetylation. A_{1320} represents absorption height of a characteristic band for –NHCO group and A_{1420} stands for the absorption height of a reference band at 1420 cm^{-1} .

Characterization: Fourier Transform Infrared (FTIR) spectroscopy (BRUKER 10033610) with attenuated total reflectance (ATR) mode was used to study the effect of different

TABLE-1 VARIATION OF DEGREE OF DEACETYLATION OF CHITOSAN WITH ALKALI CONCENTRATION									
Sample code	Sample description	Acid-base titration (%) (DD)	FT-IR spectroscopic method (%) (DD)	DD (%) in average					
CS-45%	Prepared by treating chitin with 45% KOH	40.2	37.1	38.6					
CS-55%	Prepared by treating chitin with 55% KOH	48.0	44.4	46.2					
CS-65%	Prepared by treating chitin with 65% KOH	48.7	46.5	47.6					
CS-75%	Prepared by treating chitin with 75% KOH	54.0	59.9	56.9					

treatments on the structure of the samples. The spectra were recorded in the wave number range of 4000-500 cm⁻¹ with the resolution of 10 cm^{-1} . X-Ray diffraction (XRD) measure-ments were taken from 0 to 60° with an exposure time of 400 s using a D8 advance BRUKER diffractometer with Cu target ($\lambda = 0.1541 \text{ nm}$). Solid state $^{13}\text{C CP/MAS NMR}$ spectra were measured using BRUKER spectrometer with scans 276. The XPS patterns were obtained from CSIR-NEIST, Jorhat, India using XPS XSAM-HS with a focused monochromatic MgK α X-ray source, with voltage of 12 kV, current 10 mA and 5×10^{-8} torr pressure inside the vacuum chamber. The experiment was performed to relate the effect of chitosan coating on weight loss and physical appearance of *Myrica esculenta* (kaphal) fruits.

Weight loss determination: Kaphal (*Myrica esculenta*) grown at the local farms, Tokha, Kathmandu, Nepal of uniform size with 60% or more red colour, free of physical damage and fungal infection were collected. Fruits were coated *via* dip coating; for it 100 g weight of the fruit was immersed in water and different concentration of chitosan solution (0, 0.5, 1.0, 1.5 and 2.0%) in 1% acetic acid solution at room temperature. The weight of samples was noted at the interval of 2/2 days for 12 days [16]. The weight loss percentage was calculated by using eqn. 7:

Weight loss
$$(\%) = \frac{w_0 - w_1}{w_0} \times 100$$
 (7)

where w_0 = initial weight and w_1 = final weight.

RESULTS AND DISCUSSION

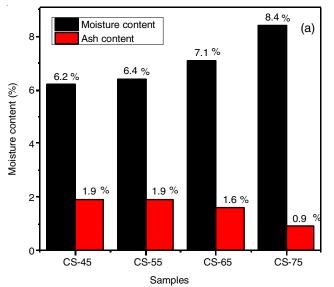
Moisture and ash content of chitosan: Moisture and ash contents of prepared chitosan as function of concentration and time varied from 6.2-8.4% and 6.2-8.6%, respectively (Fig. 1). Moisture content was found to be increased with the increase in the concentration of alkali used during deacetylation and the time of reaction. The CS-45% showed least (6.2%) percentage whereas CS-75% showed greatest (8.4%) percen-

tage of moisture content. Similarly, CS-0.5 showed the least (6.2%) percentage whereas CS-4.0 showed the greatest (8.6%) percentage of moisture content.

The prepared chitosan showed decreasing value of ash content from CS-45% (1.9%) to CS-75% (0.9%) and CS-0.5 h (2.5%) to CS-4.0 h (1.0%) with respect of concentration and reaction period of deacetylation process. The CS-75% (0.9%) and CS-4.0 h (1.0%) indicated higher purity of chitosan. Like other natural polymers, chitosan is also hygroscopic in nature due to which it showed significant moisture content [23]. It is reported that moisture content of commercial chitosan should be less than 10%. The variation in moisture content was due to difference in the intensity of sunlight, climate and relative humidity [24-26].

Ash content measurement is an indicator of the effectiveness of the demineralization step for the removal of calcium carbonate [27]. Ash content affects its solubility, average molecular weight, viscosity and other important characteristics [27]. According to No & Lee [17], the best quality of chitosan should have > 1% ash content and material as well as composition affects the ash content of chitosan [28,29]. The reported ash content of commercial chitosan and shrimp chitosan was 2.28%, moreover, purity of chitosan increases with the decrease of ash content [30]. Thus, in this work, the purity of CS-75% is highest, therefore this sample was selected for further studies.

Molecular weight of chitosan: Fig. 2 represents an average molecular weight of prepared chitosan as a function of concentration and time. It was observed that the average molecular weight of chitosan decreased with increase in concentration and deacetylation time. The CS-45% and CS-75% samples showed the highest (23000 Da) and the lowest (12000 Da) value, respectively, whereas CS-0.5 h and CS-4.0 h samples showed the highest 21000 Da and the lowest 10000 Da value, respectively. Prepared prawn shells chitosan was more depolymerized with lower molecular weight as compared to the reported values (350000 Da) and (159653 Da) [3,31]. Average molecular weight of chitosan depends on several factors such as chitin



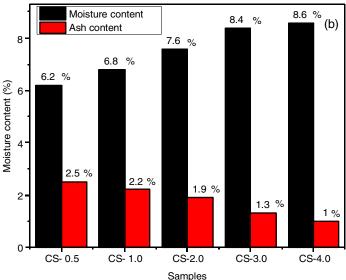


Fig. 1. Moisture and ash content of chitosan as a function of (A) KOH concentration and (B) time of deacetylation process

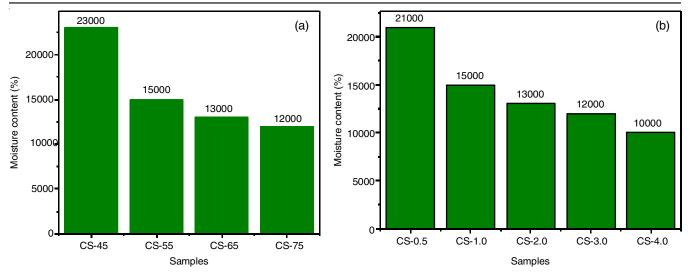


Fig. 2. Molecular weight of chitosan samples variation with (A) concentration of KOH and (B) duration of deacetylation process

sources, conditions for deacetylation process, temperature, concentration of alkali, demineralization methods, particles size, reaction time, dissolved oxygen concentration *etc.* [32].

Determination of degree of deacetylation: Degree of deacetylation of chitosan was determined *via* acid base titration [3,20] and FTIR method [21,22]. The CS-75 % and CS-4.0 h samples showed the highest degree of deacetylation as they exhibited the lowest molecular weight (Table-1). There is significant increase in degree of deacetylation with reaction time [3] and concentration in consistent with the reported value. The degree of deacetylation values are dependent on the source, purification method and the type of analytical method employed. The other factors like sample preparation, instrumental and other conditions may also influence the analysis of degree of deacetylation [20].

FTIR analysis: Each band in spectra of extracted prawn shell chitin and chitosan has been interpreted by comparing

frequencies of different vibration modes. FT-IR spectra of chitin and chitosan samples are shown in Fig. 3. A broad band ranged from 3400 to 3200 cm⁻¹ was due to OH and NH stretching vibration. The peaks at $1622 \, \text{cm}^{-1}$ is due to the stretching vibration of carbonyl group. The peak at $1553 \, \text{cm}^{-1}$ indicates NH₂ bending vibration, showing the formation of chitosan by partial deacetylation of chitin [3].

Some peaks in the range 1650-1300 cm⁻¹ indicate the vibrations of bridged C-O-C asymmetric stretching, C-O-H stretching and other ring stretching modes in both chitin and chitosan. Different bands in FT-IR spectrum of chitin (Fig. 3a) arise due to N-H, C=O, CH₃ and other complex vibrations of NHCO group. Moreover, multi bands were also observed in the range of 3400-3000 cm⁻¹ due to the NH stretching vibrations [33]. Appearance of multiple bands is due to the formation of either dimers with *cis*- or polymers with *trans*-conformation of secondary amides [20].

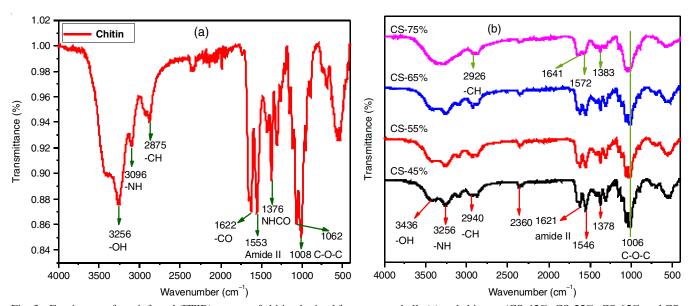


Fig. 3. Fourier-transform infrared (FTIR) spectra of chitin obtained from prawn shells (a) and chitosan (CS-45%, CS-55%, CS-65% and CS-75%) (b)

On the other hand, N-H bending vibration (amide-II) is assigned to a single strong peak which appeared at 1553 cm⁻¹. Similarly, I band, which is due to the CO stretching vibration is allotted to 1622 cm⁻¹, however, the peak is reported at 1630 cm⁻¹ [33]. It has been reported that two types of hydrogen bonds formed by amide groups in the antiparallel alignments present in a chitin crystalline region give rise to doublet in amide I band. On a contrary, chitin shows a single amide-I peak because of the parallel chain alignment present in the crystalline region [34]. In this study, amide-I band was accompanied by another peak at 1622 cm⁻¹. Further a strong, sharp band at 1376 cm⁻¹ is assigned to a complex vibration due to NHCO group, which is often known as amide-III band and the peak at frequency 1062 cm⁻¹ is interpreted as CO stretching [33,35].

Chitosan is a deacetylated derivative of chitin and therefore the major differences in FT-IR spectra lie in N-H and C=O vibrations (Fig. 3b). The position of various bands in FT-IR spectra of chitosan did not show significant shift from those of chitin. However, the band intensities showed some decrease as a decrease in vibration intensities of covalent bond due to alkali leaching [36]. Further, the analysis of spectral regions 3500-3000 and 1800-1600 cm⁻¹ is also done to evaluate any chemical change that might have occurred when chitin is deacetylated to chitosan. In chitosan, an amide group of chitin is replaced by (NH₂) group; and therefore a great interaction between O-H and NH₂ stretching vibration frequencies, compared to chitin, may result due to increased hydrogen bonding.

A broad band in the frequency range of 3500-3000 cm⁻¹ with no distinct peaks for O-H and N-H stretching was observed in the FT-IR spectra of chitosan due to increased interaction. The protonated amines in chitosan may also have caused this significant band broadening [20,35]. Moreover, compared to chitin a considerable decrease in the intensity with broadening of amide-I was observed in the frequency range 1660-1300 cm⁻¹. This could be interpreted as a decrease in the number of characteristic vibrations due to the lowering of amide-I band of chitosan. Further, coupling of NH bending vibrations of NH₂ group in chitosan with amide-II band may account for the broad and weak band in the frequency range 1649-1377 cm⁻¹ [33,35]. However, characteristic amide peaks are not com-

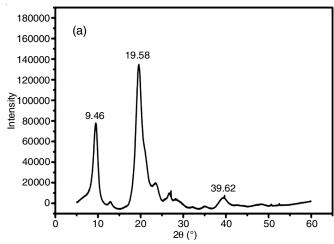
pletely lost that means the chitin is only partially deacetylated.

XRD analysis: The XRD patterns of the extracted chitin and its derivative chitosan (CS-75%) are shown in Fig. 4. The broad diffraction peaks at $2\theta = 10^{\circ}$ and 20° as typical finger prints of semi-crystalline chitosan have been reported [37]. Prepared chitosan (CS-75%) contains major peaks at 9.5° and 19.6°. According to Feng et al. [38] WAXD patterns of shrimp chitosan showed two major characteristic peaks at 20 are 10.6° and 20.4°, which are assigned as the fingerprints of semicrystalline nature of chitosan. Chitin also shows the measurable peaks at the region of 19-21°, which indicates that chitin is more crystalline than chitosan. The crystalline size of chitin and chitosan (CS-75%) were found as 3.8 and 2.8 nm respectively, calculated by using Debye Scherrer's formula *i.e.* $D = n\lambda/\beta \cos \theta$ θ ; where, D is crystalline size, n = 0.9 (constant), λ = 0.154 nm for copper target, β = full width at half maxima (FWHM) and β = Bragg's angle.

¹³C NMR analysis: Fig. 5 presents the ¹³C NMR spectra of selected chitosan sample (CS-75%) and the chemical shifts are also assigned. Chitosan shows the major peaks similar to those reported in literature [38,39]. Signal at 24 ppm corresponds to methyl group (-CH₃) and signals at 57 ppm (C2), 61 ppm (C6), 76 ppm (C3 and C5), 83 ppm (C4), 103 ppm (C1), 174 ppm (C=O) represent carbon of chitosan.

Feng *et al.* [38] found that the solid state ¹³C CP-MAS NMR spectra of shrimp chitosan and crab chitosan showed the major characteristic peaks (Table-2). After analysis of ¹³C NMR spectra of chitosan from various sources including the crab shell chitosan, shrimp shell chitosan and chitin, the chemical shifts of C-1 and C-4 carbon in 1,4-linked carbohydrates are supposed to be highly sensitive towards the conformational change at the glycosidic linkage [39]. The appearance of signals for -CH₃ (24 ppm) and C=O (174 ppm) attributed to carbon from acetamido moiety of chitin in the chitosan [40] shows that the chitosan derived from chitin is partially deacetylated.

X-ray photoelectron analysis: Extracted chitin characterized by using XPS spectrometer contains three major peaks (Fig. 6). The XPS result of chitin observed C 1s peak at 286 eV represent the C-C binding energy. The N 1s peak at 400 eV



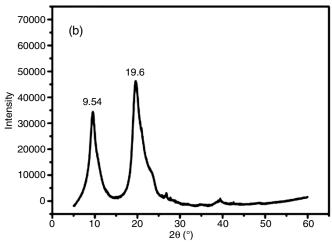
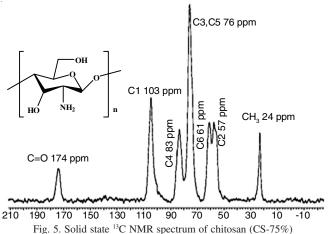
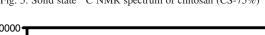


Fig. 4. X ray diffractogram of (a) chitin and (b) chitosan (75%)

TABLE-2 CHEMICAL SHIFTS OF CHITIN AND CHITOSAN OBTAINED FROM ¹³ C NMR (ppm FROM TMS)											
Source	C=O	C-1	C-4	C-5, C-3	C-6	C-2	CH ₃	Ref.			
Chitin	173.8	104.1	83.0	75.7, 73.3	60.8	55.2	22.8	[39]			
Commercial chitosan	-	104.7	85.7, 81.0	74.1	60.7, 59.6	56.8	-	[39]			
Crab shell	-	105.0	85.6, 81.2	75.5	60.3	56.4	-	[38]			
Prawn shell synthesized sample (CS-75%)	174	103	83	76	61	57	24				





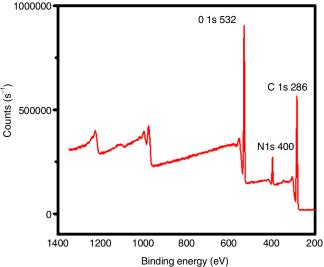


Fig. 6. X-ray photoelectron spectra (XPS) of chitin isolated from prawn shells

assigned to binding energy in amino group. The O 1s peak at 532 eV describes to C-O or O-H binding energy. Yap *et al.* [41] confirmed the presence of carbon, nitrogen and oxygen in crosslinked chitosan thin film by using XPS. The peaks observed were: C 1s peak at 285.0 eV for C-C chemical binding, 286.6 eV for C-O, C-N or C-O-C and 288.2 eV for C=O or O-C-O chemical bindings.

Effects of chitosan coating on weight loss of Myrica esculenta during storage: Shelf life of Myrica esculenta fruit was analyzed via chitosan coating on fruits and the shelf life was inspected by the change in their physical appearance and weight loss method. All samples demonstrated a gradual loss of weight during storage (Fig. 7). Throughout storage, the loss of weight of uncoated fruit was significantly greater than that of

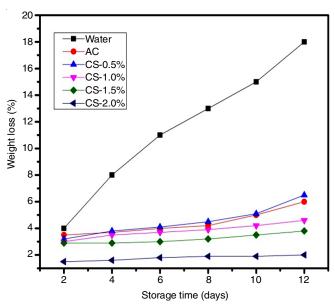


Fig. 7. Effects of chitosan coating on weight loss of Myrica esculenta fruits

coated fruit. Effect of chitosan coating in weight loss of fruit indicated that there was gradual increase in weight loss in pure water, whereas there was significant decrease in weight loss in 1% acetic acid solution. Similarly, there was only a marginal difference in weight loss of fruit matter was observed in 0.5%, 1% chitosan solution and in acetic acid solution. However, in 1.5% and 2.0% chitosan solution there was slightly slower rate of weight loss than the others. About 2.0% chitosan coating showed the highest beneficial effect on weight loss of the fruit than the fruit in other solutions. The greater viscosity of the 2.0% chitosan solution has been shown to result in a coating of greater thickness, further reducing weight loss. The concentration of chitosan in the coating solution affects the fungal decay of the fruit. A concentration of chitosan inhibits fungal growth during the storage period of strawberry [42]. This effect has been reported for numerous horticultural commodities such as tomatoes, strawberries, longan, apples, mangoes, bananas, bell peppers, etc. [16,43,44]. Shelf life extension of litchi fruits by chitosan coating combined with ascorbic acid can be used for litchi fruits storage in commercial scale [45].

Chitosan has been reported to be more effective at delaying weight loss in banana and mango and strawberries than starch and cellulose derivatives. Chitosan has the capacity to inhibit growth of several fungi, to induce chitinase and to elicit phytoalexins in the host tissues. Thus, the control of decay in strawberries could be attributed to either the fungistatic property of chitosan or to its ability to induce defense enzymes and phytoalexins in plants or a combination [43]. Weight loss of fruit is mostly related with respiration and moisture evaporation through

the skin. The thin skin of Kaphal fruits makes them susceptible to rapid water loss, resulting in shriveling and deterioration. The rate at which water is lost depends on water pressure gradient between the fruit tissue and the surrounding atmosphere and the storage temperature [42]. Edible coatings of chitosan act as barriers and hence decrease dehydration. This study showed that the beneficial effect of chitosan can be increased when the polymer was applied at a greater concentration.

Effect of chitosan coating on colour of *Myrica esculenta* (Kaphal) fruit: As shown in Fig. 8, colour quality of Kaphal fruit decreased as the period of storage increased. After 8 days of storage, the initial bright colour of the fruit had largely disappeared and rotten. Compared with water, 0.5% chitosan, 1.0% chitosan and 1.5% chitosan, 2.0% chitosan remained almost unaffected with bright colour and about 80% of it did not rot until 12 days after storage. In this study, increasing concentrations of chitosan were generally more effective for longer storage.



Fig. 8. Effects of chitosan coating on colour change of *Myrica esculenta* fruits

Conclusion

Chitin was successfully isolated from the prawn shells and also deacetylated into chitosan. The effect of concentration of alkali (KOH) and the time of reaction on to the process of deacetylation was studied. The rate of chitosan formation was found to increase with increase in reaction time and KOH concentration. The FT-IR spectra provided the insights into the structural features of chitin and chitosan. Both chitin and chitosan essentially containing the hydrogen bond could be distinguished though the nature of absorption bands in the frequency region of O-H and N-H stretching and amide group vibrations. The X-ray diffraction study showed the semi-crystalline nature of chitosan and more crystallinity of chitin than chitosan. The XPS spectra confirmed the presence of the reported amount of carbon, nitrogen and oxygen in chitin. The ¹³C NMR spectra

showed partial deacetylation of chitin into chitosan with the carbon atoms of essential functionalities in polymer chain. Chitosan treatment was found beneficial to reduce the adverse effects of chemical residues on Kaphal fruit. The preservative coatings of chitosan on the fruit matter showed a remarkable potential to prolong the storage or shelf life, control the decay and reduce the weight loss of *Myrica esculenta* fruits. This study can be extended by checking the quality of fruits after chitosan coatings.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Dr. Paras Nath Yadav and Prof. Dr. Rameshwar Adhikari, Central Department of Chemistry, Tribhuvan University, Nepal; Prof. A.R. Chakravarty, Indian Institute of Science, Bangalore, India; and Dr. Biswajit Sah, Research-North East Institute of Science & Technology (CSIR-NEIST), Jorhat, India for their guidance and support to this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- P.K. Dutta, J. Dutta and V.S. Tripathi, J. Sci. Ind. Res. India, 63, 20 (2004).
- S. Pokhrel, P.N. Yadav and R. Adhikari, Nepal J. Sci. Technol., 16, 99 (2016);
 - https://doi.org/10.3126/njst.v16i1.14363
- S. Pokhrel, R. Lach, W. Grellmann, A. Wutzler, W. Lebek, R. Godehardt, P.N. Yadav and R. Adhikari, Nepal J. Sci. Technol., 17, 5 (2016); https://doi.org/10.3126/njst.v17i1.25056
- N. Gagné and B.K. Simpson, Food Biotechnol., 7, 253 (1993); https://doi.org/10.1080/08905439309549861
- S. Pokhrel and P.N. Yadav, J. Macromol. Sci. A, 56, 450 (2019); https://doi.org/10.1080/10601325.2019.1581576
- M.K. Jang, B.G. Kong, Y.I. Jeong, C.H. Lee and J.W. Nah, *J. Polym. Sci. A Polym. Chem.*, 42, 3423 (2004); https://doi.org/10.1002/pola.20176
- H. Sashiwa and S.-I. Aiba, Prog. Polym. Sci., 29, 887 (2004); https://doi.org/10.1016/j.progpolymsci.2004.04.001
- 8. L. Qi, Z. Xu, X. Jiang, C. Hu and X. Zou, *Carbohydr. Res.*, **339**, 2693 (2004);
 - https://doi.org/10.1016/j.carres.2004.09.007
- K. Murakami, H. Aoki, S. Nakamura, S. Nakamura, M. Takikawa, M. Hanzawa, S. Kishimoto, H. Hattori, Y. Tanaka, T. Kiyosawa, Y. Sato and M. Ishihara, *Biomaterials*, 31, 83 (2010); https://doi.org/10.1016/j.biomaterials.2009.09.031
- D. Jianglian and Z. Shaoying, J. Food Process. Technol., 4, 227 (2013); https://doi.org/10.4172/2157-7110.1000227
- H.S. Adhikari and P.N. Yadav, Int. J. Biomater., 2018, 1 (2018); https://doi.org/10.1155/2018/2952085
- T. Chandy and C.P. Sharma, Biomat. Art. Cells Art. Org., 18, 1 (1990); https://doi.org/10.3109/10731199009117286
- S. Rawat, A. Jugran, L. Giri, I.D. Bhatt and R.S. Rawal, Evid. Based Complement. Alternat. Med., 2011, 1 (2011); https://doi.org/10.1093/ecam/neq055
- Y.S. Gusain and V.P. Khanduri, Ecol. Environ. Conserv., 22, S267 (2016).
- R.K. Patel and L.C. De Sohphie, *ENVIS Bull. Himal. Ecol. Dev.*, 14, 34 (2006).
- Y. Jiang and Y. Li, Food Chem., 73, 139 (2001); https://doi.org/10.1016/S0308-8146(00)00246-6
- 17. H.K. No and M.Y. Lee, J. Korean Soc. Food Sci. Nutr., 24, 105 (1995).

- L.E. Allison and C.D. Moodie, in eds: C.A. Black, Methods of Soil Analysis, Part 2, Agronomy 9, Madison, Wisconsin, p. 671 (1965).
- T.D. Jiang, Chitosan; Chemical Industry Press: Beijing, China, pp 91-100 (2001)
- Y. Yuan, B.M. Chesnutt, W. Haggard and J.D. Bumgardner, *Materials*, 4, 1399 (2011); https://doi.org/10.3390/ma4081399
- J. Brugnerotto, J. Lizardi, F.M. Goycoolea, W. Argüelles-Monal, J. Desbrières and M. Rinaudo, *Polymer*, 42, 3569 (2001); https://doi.org/10.1016/S0032-3861(00)00713-8
- S. Ghimire, B. Neupane, S. Pokhrel, H.H. Le, W. Lebek, G. Heinrich, P.N. Yadav and R. Adhikari, *Polym. Res. J.*, 11, 1 (2017).
- T.A. Khan, K.K. Peh and H.S. Ch'ng, J. Pharm. Pharm. Sci., 5, 205 (2002).
- H. Tajik, M. Moradi, S.M.R. Rohani, A.M. Erfani and F.S.S. Jalali, *Molecules*, 13, 1263 (2008); https://doi.org/10.3390/molecules13061263
- M. Hossain and A. Iqbal, J. Bangladesh Agric. Univ, 12, 153 (2014); https://doi.org/10.3329/jbau.v12i1.21405
- P. Premasudha, P. Vanathi and M. Abirami, *Int. J. Sci. Res.*, 6, 1194 (2017).
- S.O. Fernandez-Kim, Master Thesis, Louisiana State University and Agricultural and Mechanical College, p 1338 (2004).
- Q. Li, E.T. Dunn, E.W. Grandmaison and M.F.A. Goosen, *J. Bioact. Compat. Polym.*, 7, 370 (1992); https://doi.org/10.1177/088391159200700406
- 29. S. Pokhrel, Ph.D. Thesis, Tribhuvan University, Kathmandu, Nepal (2017).
- V. Mohanasrinivasan, M. Mishra, J. Paliwal, S. Singh, E. Selvarajan,
 V. Suganthi and C.S. Devi, *3 Biotech*, 4, 167 (2014);
 https://doi.org/10.1007/s13205-013-0140-6
- S. Paul, A. Jayan, C.S. Sasikumar and S.M. Cherian, *Asian J. Pharm. Clin. Res.*, 4, 201 (2014).
- M. Laka and S. Chernyavskaya, Proc. Estonian Acad. Sci. Chem., 55, 78 (2006)
- S. Pokhrel, R. Adhikari and P.N. Yadav, Asian J. Chem., 29, 1602 (2017); https://doi.org/10.14233/ajchem.2017.20612

- R. Shelma, W. Paul and C.P. Sharma, Trends Biomater. J. Artif. Organs, 22, 111 (2008).
- 35. V. Coma, A. Deschamps and A. Martial-Gros, *J. Food Sci.*, **68**, 2788 (2003);
 - https://doi.org/10.1111/j.1365-2621.2003.tb05806.x 6. S. Minami, H. Suzuki, Y. Okamoto, T. Fujinaga and Y. Shigemasa,
- S. Minami, H. Suzuki, Y. Okamoto, T. Fujinaga and Y. Shigemasa, *Carbohydr. Polym.*, 36, 151 (1998); https://doi.org/10.1016/S0144-8617(98)00015-0
- C. Peniche, W. Argüelles-Monal and F.M. Goycoolea, Monomers, Polymers and Composites from Renewable Resources, Elsevier, p. 517 (2008).
- F. Feng, Y. Liu, B. Zhao and K. Hu, *Procedia Eng.*, 27, 718 (2012); https://doi.org/10.1016/j.proeng.2011.12.511
- L. Heux, J. Brugnerotto, J. Desbrieres, M.F. Versali and M. Rinaudo, Biomacromolecules, 1, 746 (2000); https://doi.org/10.1021/bm000070y
- M.K. Yadav, S. Pokhrel and P.N. Yadav, J. Macromol. Sci. A, 57, 703 (2020); https://doi.org/10.1080/10601325.2020.1763809
- W.F. Yap, W.M.M. Yunus, Z.A. Talib and N.A. Yusof, *Int. J. Phys. Sci.*, 6, 2744 (2011); https://doi.org/10.5897/AJBM11.121
- P. Hernández-Muñoz, E. Almenar, V.D. Valle, D. Velez and R. Gavara, Food Chem., 110, 428 (2008); https://doi.org/10.1016/j.foodchem.2008.02.020
- F. Kittur, N. Saroja, Habibunnisa and R. Tharanathan, *Eur. Food Res. Technol.*, 213, 306 (2001); https://doi.org/10.1007/s002170100363
- A.R. Al Eryani, T.M.M. Mahmud, S.R. Syed Omar, A.R. Mohamed Zaki and H.I. Ali, Asian J. Microbiol. Biotechnol. Environ. Sci., 10, 219 (2008).
- C. Sun, W. Du, X. Cheng, X. Xu, Y. Zhang, D. Sun and J. Shi, *Afr. J. Biotechnol.*, 9, 825 (2010); https://doi.org/10.5897/AJB09.1253