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Preperation of nanochitosan from radiation degraded oligochitosan for shelf life extension of strawberry

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Abstract: Oligochitosans (OCT) were prepared from chitosan (CTS) by gamma irradiation technique. The parameters affecting to the chitosan degradation were studied. And then, OCT nanoparticles wereformed using the method of tripolyphosphate (TPP) cross-linking. Effect of concentration and molecular weight of OCT, concentration of TPP on particle size of the formed OCT nanoparticles were also studied. The formation of OCT nanoparticles was verified by Fourier transform infrared (FT-IR) spectrometer and differential scanning calorimeter (DSC), the morphology was observed using scanning electron microscope (SEM), and the characteristics (particle size and zeta potential) of OCT nanoparticles were also studied. The effect of OCT nanoparticles on strawberry presevation was carried out using the coating method. Results showed that an increase in radiation dose resulted in a decrease of chitosan molecular weight. The OCT with molecular weight of approximately 7.7 kDa was obtained by the synergistic effect of hydrogen peroxide (5 %, v/v) and gamma ray at dose of 30 kGy. The smaller OCT nanoparticles was obtained with a lower molecular weight of OCT. The results of FTIR, DSC indicated the success in the formation of OCT nanoparticles with the particle size approximately 129.9 nm, with the spherical shape. The application of OCT nanoparticles on strawberry has prolonged the preservation times approximately 2.5 times higher compared to the control.

Keywords: *Nano oligochitosan, Radiation degradation, Synergistic action, Shelf-life extension, Strawberry.*

I. INTRODUCTION

Nowadays, consumption of fresh fruits and vegetables has attracted increasing attention due to their high nutritional values. Nevertheless, the major problem is their perishable nature resulting many troubles such as weight loss, fungal decay… For that reason, the maintainance of their quality for a longertime (preservation) is necessary. Many techniques have been studied to extend the shelf life of fresh produces such as low temperature, controlled and modified atmosphere packaging. On the other hand, there are many studies using chitosan and oligochitosan to preserve post-harvest agricultural products. The use of CTS or OCT to form the physical membrane surrounding the agricultural products will prevent their weigh loss. In addition, this physical membrane also inhibits the bacteria growth due to the antibacterial properties of CTS or OCT and inhibition of oxidation of the postharvest agricultural products [2].

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Nanotechnology has been extensively researched and applied in many fields such as agriculture, cosmetics, food and medicine [1]. Recently, the CTS nanoparticle dispersion was used to replace for chitosan solution due to its higher effect. Particularly, the direct antimicrobial activity of CTS nanoparticle. dispersion was 80-100 times longer than that of chitosan solution.

The purpose of this investigation is the shelf-life extension of agricultural products using nano oligochitosan. Firstly, oligochitosan was prepared by synergistic effect technique. Parameters affecting the degradation of CTS were investigated. OCT nanoparticles were prepared using ionic gelation method. The formation of OCT nanoparticles was demonstrated by the FTIR and DSC methods. The morphology and particle size of nano CTS were studied. Preservation efficiency were evaluated on strawberry.

II. EXPERIMENTAL

A. Materials and methods

Strawberry collected in the garden. CTS molecular weight of 101.9 kDa, derived from crab shell was purchase from Chitosan Viet Company, (Vietnam), acid acetic and sodium triphosphate pentabasic (TPP) were purchased from Sigma-Aldrich. All the other reagents used in the experiments were of analytical grade. Distilled water was used for the experiment.

- *Preparation of oligochitosan by synergistic effect technique* [8]

Oligochitosan was prepared using the method reported by Hien et al (2012). Firstly, chitosan was swelled in 25ml of hydrogen peroxide solution 5% for 1 hour then irradiated with Gamma Chamber 5000 irradiator at doses of 0-30 kGy, dose rate 2.4kGy/h. Determine the average molecular weight by GPC.

- *Preparation of chitosan nanoparticles*

CTS-TPP nanoparticles were prepared using the method reporting previously [6]. CTS, 1mg/mL was dissolved in 1% (w/v) acetic acid and sonicated for 60 minutes.Simultaneously, TPP solution was prepared at a concentration of 1 mg/mL. CTS-nanoparticles were formed by adding TPP solution into CTS solution under mechanical stirring (1000 rpm) at room temperature [10, 11].

- *Characterization of chitosan and chitosan nanoparticles*

The molecular weight (M_w): The molecular weight of degraded chitosan was determined by LC – 20AD gel permeation chromatography (GPC) (Shimadzu, Japan) with detector RID 20A and the columns SB-803 HQ from Shodex (Japan). The standards for calibration of the columns were pullulan (M_w 12.2 – 100× 10^3 Da). The eluent was aqueous solution 0.25M CH_3COOH/0.25M CH_3COONa with the flow rate of 0.5 ml min^{-1} and temperature at 40^0C [1]. The chitosan sample concentration was 0.1% (w/v).

Fourier transform infrared spectroscopy (FT-IR) analysis: FT-IR analysis of chitosan and chitosan nanoparticles were performed between 400and 4000 cm^{-1} at 2 cm^{-1} using FT/IR 4600 spectrometer (Jasco, Japan).

The degree of deacetylation was calculated based on IR spectra according to the following Eq.(1) [5]:

$$DDA\ \% = 100 - [(A_{1320}/A_{1420} - 0.3822)/0.03133] \quad (1)$$

Where A_{1320} and A_{1420} are absorbances of chitosan at 1320 cm^{-1} and 1420 cm^{-1}, respectively.

Differential scanning calorimetry (DSC): Differential scanning calorimetry measurements were performed in a DSC-60 (Shimazu, Japan). The DSC curves were performed under dynamic nitrogen atmosphere (50–100 mL min^{-1}) using sample mass is 4 mg

and heating rates is 20 ◦C min^{-1}. Accurately weighed samples (±0.1 mg) were placed into a covered aluminum sample holder with a central pin hole. An empty sample holder was used as reference and the runs were performed by heating the samples from 30 up to 600^0C.

Particle size and zeta potential: The measurements of particle size and zeta potential of nanoparticles were performed using a Nano Particar ZS-100 (HORIBA, Japan) on the basis of Dynamic light scattering (DLS) techniques at an angel of 90º.

- *Coating process*

The fruits were coated by dipping method. The experiment was arranged in a completely randomized design and consisted of 1 coating in 2 minutes and 4 storage times (1, 2, 4 and 7 days), with three replicates. The experimental unit consisted of ten strawberry. Coating was followed with cool air drying (20-30˚C) then stored at room temperature. To evaluate the changes in quality of the coated samples, the criteria of weight loss and fungal decay was studied at interval time points.

Weight loss: weight loss was calculated according to the weight of each sample before and after storage and expressed as the percentage weight loss compared to the initial weight.

Fungal decay: Fungal decay was visually inspected during the storage period. Results were expressed as the percentage of fruits infected. The fungal decay can be obtained from the Eq. (2).

$$Fungal\ Decay\ \% = \frac{\text{Number of fruit decay}}{\text{The total number of fruit}} \times 100 \quad (2)$$

- *Statistical analysis*

Statistical analysis of data was performed using SPSS software package version 16.0 by one-way analysis of variance (ANOVA), assuming confidence level of 95% ($P<0.05$) for statistical significance. All analysis was performed in triplicate.

B. Results and discussion

Investigating the chitosan degradation effect, we firstly investigated some parameters of initial chitosan such as deacetylation, molecular weight M_w and Polydispersity Index PI. In which, deacylation determined using the FTIR spectra; Molecular weight M_w indicated average molecular weight of CTS and determined using GPC; PI indicated polydispersity index of CTS and determined using GPC.

- *IR spectral analysis*

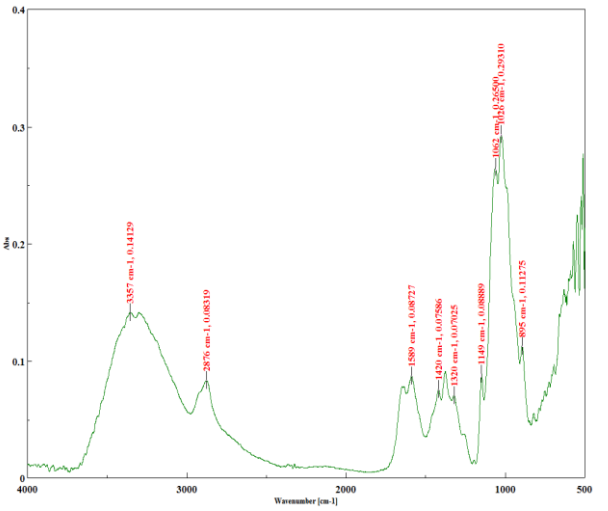

Fig.1. IR spectrum of chitosan.

The bands 3290 cm^{-1} is determined by υ(OH) overlapped on υs(N–H). The band from 2867 cm^{-1} is determined by υ(–C=O) of the amide group CONHR of the chitosan. The bands 1568 cm^{-1} (chitosan) are determined by υ(–C=O) of the proton amide group, and δ(NH_3) is determined by the proton amide group. The bands 1417 cm^{-1} is determined by δ(OH). The bands 1372cm^{-1} chitosan are determined by δ(–CH_3). The bands 1318 cm^{-1}

is determined by υs(-CH_3) third amide ω (–CH_2) + OH deformation in plane. The bands 1152 cm^{-1} is determined by υas(C=O) oxygen bridges resulting from the deacetylation of the chitosan. The bands 1060 cm^{-1} is determined by υ(C=O) by the bindings C–O–H, C–O–C and CH_2CO. The bands 892 cm–1 is determined by ω(C–H) from the polysaccharide's structure (Fig.1).

The degree of deacetylation was calculated according to the following equation

$$DDA\ \% = 100 - [(A_{1320}/A_{1420} - 0.3822)/0.03133]$$

The result shows that, DDA of initial chitosan is 82.64%.

- *GPC analysis.*

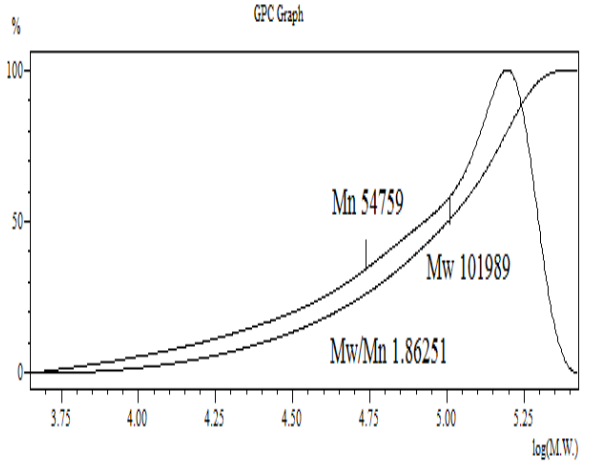

Fig.2. GPC chromatogram of initial chitosan.

The molecular weight (M_w) of degraded chitosan was determined by GPC with pullulan as a standard. The chromatogram shown that molecular weight (Mw) of initial chitosan is 101.9 kDa, Mn is 54.7 and PI is 1.86 (Figure 2).

- *The effect of irradiation conditions on the degradation of chitosan swelled in hydroperoxide*

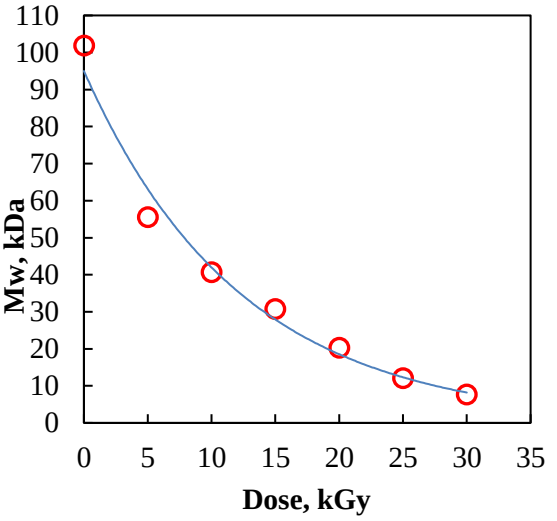

Fig.3. The molecular weight of chitosan versus dose.

The effect of absorbed (irradiation) dose on the radiation cleavage yield of chitosan swelled in hydroperoxide solution is shown in Figure 3. The results show that the average molecular weight (M_w) of chitosan decreases with radiation dose. Particularly, M_w is approximately of 12.1 kDa and 7.7 kDa at absorbed dose of 25 kGy and 30 kGy, respectively.

- *Effect of CTS/TPP mass ratio onto chitosan nanoparticle size*

In this study, we investigated the effect of CTS/TPP ratio (w/w) onto the particle size of nano chitosan with Mw ~101 kDa at initial concentration 0.1%. The results are shown in Figure 4.

The concentrations of CTS and TPP solutions and ratio of CTS to TPP by weight have an important effect on the formed CTS nanoparticles. As the TPP concentration was increased, the reactive product changed in three stages [13]

Stage 1:When the TPP concentration is small, the mixture was transparent.

Stage 2: As the TPP concentration increases gradually, the mixture became opaque due to the formation of CTS nanoparticles.

Stage 3: When the TPP concentration is too high the precipitation was happened due to the formation of very large CTS particles or the clump of CTS particles.

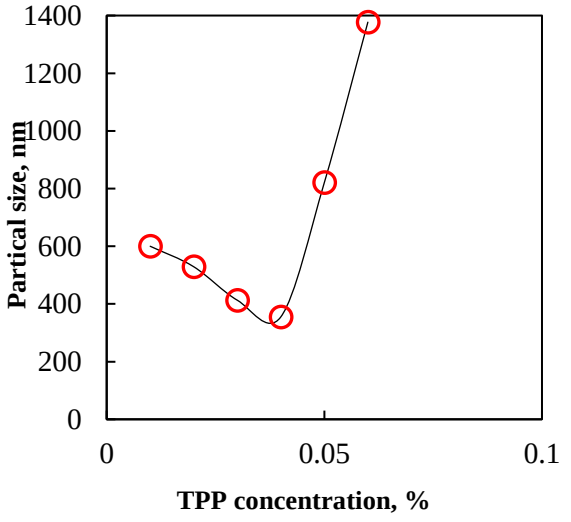

Fig.4. The particle size of nanochitosan versus TPP concentration.

When TPP concentration was very low, the number of phosphate groups was not enough to produce effective electrostatic attraction with the amino groups of CTS [12]; therefore, the solution is still transparent because there was not the formation of CTS nanoparticle. As the TPP content was increased gradually, the solution became opaque and when the CTS/TPP mass ratio reached to ratio of 5:2 (w/w), particle size of CTS nanoparticles was about 349 nm. Thereafter, when TPP concentration was very high, the larger particles were formed and the precipitation was happened probably due to the coagulation of the excessive CTS nanoparticles.

- *Effect of M_w chitosan on chitosan nanoparticle size and zeta potential*

Based on the results of *Effect of CTS/TPP mass ratio onto chitosan nanoparticle size*, CTS:TPP ratio of 5:2 (w/w) was chosen to investigate the effect of chitosan molecular weights on characteristics of the formed nanoparticles. Effect of M_w chitosan on chitosan nanoparticle size and zeta potential shown in Fig.5. The smaller particle size and higher zeta potential were achieved when the lower molecular weight of CTS was used. It is because the smaller particle size led to the higher charge density resulted in the higher value of zeta potential.

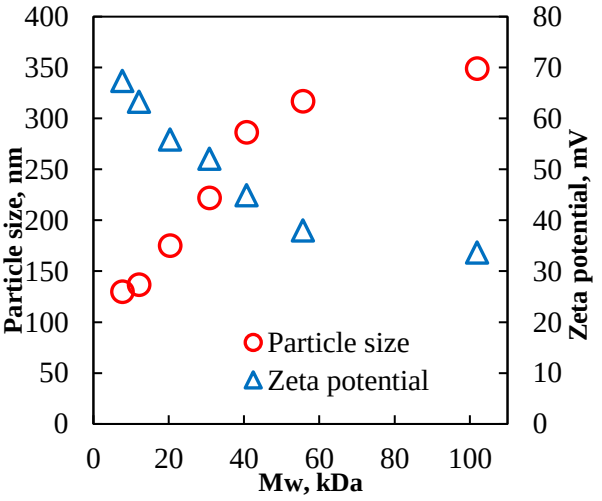

Fig.5. Effect of M_w chitosan onto chitosan nanoparticle size and zeta potetial

- *Characterization of nano chitosan*

IR: Figure 6 shows the IR absorption spectra of chitosan nanoparticle (a); chitosan (b) and TPP (c)

It can be seen that, two spectra, 6a and 6b, had similar peaks, locations, and intensities. In Figure 6b, at 1584 cm^{-1}, there was an $-NH_2$ absorption peak. While in Figure 6a, besides the same absorption peak found in Figure 6b, it can be observed that an $–NH_2$ stretch vibration absorption peak drifted to a low wave number in 6a at 1534 cm^{-1}, this indicated that the phosphate group linked to the

amino group and formed strong intermolecular and intermolecular hydrogen bond.

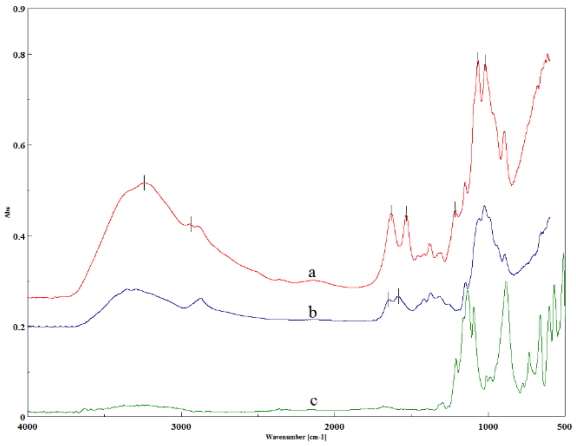

Fig.6. IR spectrum of (a) chitosan nanoparticles; (b) chitosan and (c)TPP.

DSC: Fig.7. shows DSC curves of (a) chitosan and (b) chitosan nanoparticles.

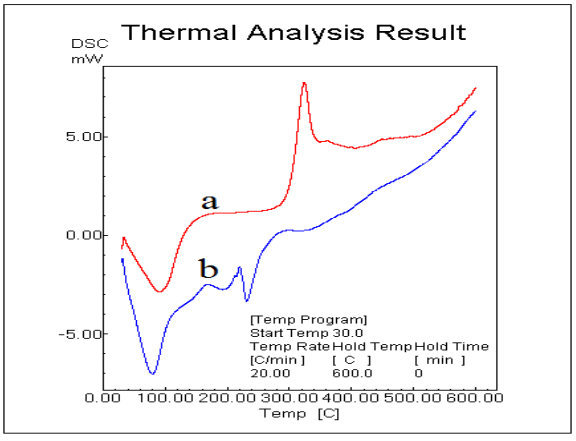

Fig.7. DSC curves of (a) chitosan and (b) chitosan nanoparticles.

Figure 7a shows a wide endothermic peak at 90^0C which is attributed to the elimination of absorbed water and a sharp exothermic peak at 324^0C which is due to the decomposition of chitosan chains. The DSC curve of chitosan nanoparticles fig. 7b has a wide enothermic peak below 90^0C which is due to the removal of absorbed water and a sharp endothermic peak at 268^0C associated with the breakage of chitosan phosphoric acid cross linkage. The decomposition of chitosan nanoparticles is expected to happen well above 600^0C. Decreased crystallinity indicates change in solid state structure of chitosan due to crosslinking.

FE-SEM, particle size distribution and zeta potential of OCT nanoparticles

The results of the FE-SEM image of the nano chitosan shown in Figure 8 shown that the morphology of OCT nanoparticles is spherical and relatively uniform.

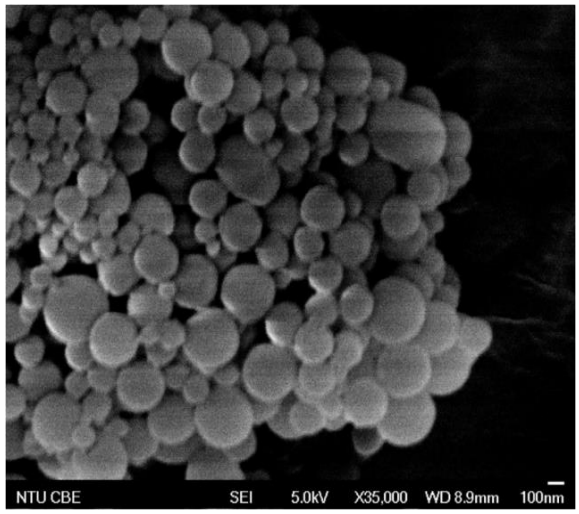

Fig.8. FE-SEM image of the OCT nanoparticles

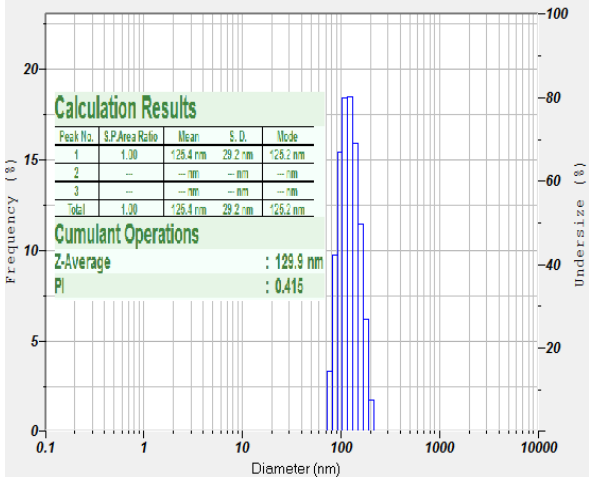

Fig.9. Particle size distribution of OCT nanoparticles

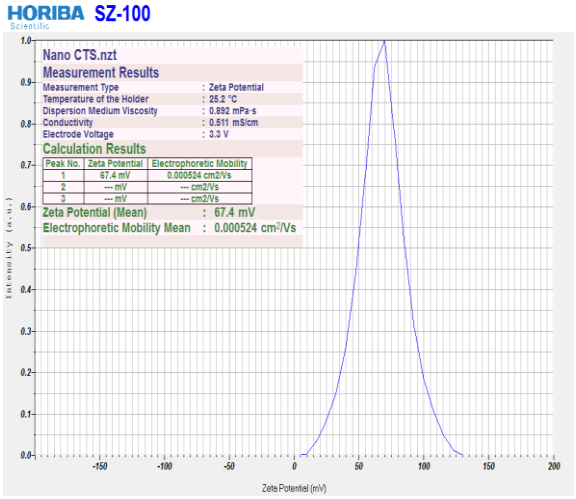

Fig.10. Zeta potential of OCT nanoparticles 129.9 nm

In addition, as shown in Fig. 9 and Fig. 10, the particle size and zeta potential of the OCT nanoparticles approximately 129.9 nm and +67.4 mV, respectively. On the other hand, the Fig. 9 also show the particle size of OCT nanopartices is very uniform due to the narrow distribution of the peak.

- Shelf-life extension of strawberry using OCT nanoparticles

The fruits were randomly harvested at the commercial ripening stage and screened for uniformity and the absence of physical defects or decay. Subsequently, the strawberry fruits were randomly distributed into four groups prior to treatment with three replicates. Control group were dipping in water, TPP group were dipping in 200 ppm TPP solution, OCT group were dipping in 500 ppm OCT solution and OCT nanoparticle group were dipping in 500 ppm nano oligochitosan solution with the particle size approximately 129.9nm. The results are shown in table. I.

Table I. Weight loss and fungal decay of strawberry versus time.

	Days	Control	TPP	OCT	OCT nanoparticle
Weight loss (%)	**1**	8.83 ± 0.24^{b}	9.36 ± 0.09^{b}	2.23 ± 0.14^{a}	2.10 ± 0.15^{a}
	2	13.06 ± 0.43^{b}	15.30 ± 0.23^{c}	3.46 ± 0.14^{ab}	3.00 ± 0.15^{a}
	4	22.40 ± 0.73^{c}	25.80 ± 0.20^{d}	7.60 ± 0.34^{b}	5.63 ± 0.14^{a}
	7	25.60 ± 0.46^{c}	28.73 ± 0.49^{d}	11.93 ± 0.34^{b}	9.63 ± 0.26^{a}
Fungal decay (%)	**1**	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	2	1.66 ± 1.66^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	4	8.33 ± 3.33^{b}	6.66 ± 1.66^{ab}	1.66 ± 1.66^{ab}	0.00 ± 0.00^{a}
	7	11.66 ± 1.66^{c}	10.00 ± 2.88^{b}	5.00 ± 2.88^{ab}	1.66 ± 1.66^{a}

Different letters in the same raw indicate significant difference ($P<0.05$)

Fruit weight loss is mainly associated with respiration and moisture evaporation through the skin. The thin skin of strawberry makes them susceptible to rapid water loss, resulting in shrivelling and deterioration making fruit surface wounded. Strawberry preservation capacity of 500 ppm nano oligochitosan solution with particle size of 129.9 nm at room temperature is presented in Table I. The results show that in the control sample, the weight loss and the fungal decay of strawberry after 7 days was very high. Weight loss is 25.6% and fungal decay is 11.66%. This also occurred similar to the sample coating with TPP solution. With sample coating by OCT after 7 days, weight loss is 11.93% but after 4 days the fungal decay were happend. With sample coating by CTS nanoparticle 129.9nm after 7 days, weight loss of strawberry is 9.63% and the fungal decay is very low about 1.66%. In fact, nano chitosan coating acts as a semi-permeable barrier to

water, resulting in procrastination of water transfer and more control over weight loss.

III. CONCLUSIONS

Oligochitosan was prepeared by synergistic effect technique with gamma ray and hydro peroxyt 5%. Molecular weight of oligochitosan is 7.7 kDa. Molecular weight of chitosan decreases as the dose increases. Nano oligochitosan particle size 129.9 nm was prepared from oligochitosan by ionic gelation technique with TPP. The lower the CTS molecular weight, the smaller the particles derived from the chitosan. More valuable effect of chitosan nanoparticle was that the chitosan concentration of 500 ppm could significantly affect the qualities including fungal decay, water loss. Nano-chitosan coating delayed softening and ripening, changes in weight loss, fungal decay. Chitosan nano-particle has high efficiency in extending the shelf life of strawberry after 7 days at room temperature.

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