

## Antioxidative properties of irradiated chitosan/vitamin C complex and their use as food additive for lipid storage

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**ABSTRACT:** Improving the antioxidant activity of chitosan was achieved by decreasing their molecular weight by  $\gamma$  rays followed by incorporation with vitamin C to prepare chitosan/vitamin C (CSVC) complex in the range of nanoparticles. Transmittance electron microscopy of CSVC complex showed mean diameters ranged from 23.2 to 82 nm. The antioxidant activities of CSVC complexes were examined using scavenging effect on DPPH radicals and reducing power measurements. CSVC complexes have a synergistic effect on increasing the antioxidant properties rather than their individual effects. The effect of CSVC complexes on lipid peroxidation of meat during 21 days of refrigerated storage was investigated using thiobarbituric acid reactive substance (TBARS) assay. Treatment of meat with CSVC complex reduced lipid peroxidation about 75% after 7 days of storage as a result the decrease in TBARS values. The results demonstrate promising use of CSVC complex as antioxidants for lipid storage. © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 2015, 132, 42105.

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### INTRODUCTION

One of the most important factors that influence the quality and acceptance of meat and poultry during refrigerated or frozen storage is lipid oxidation. The oxidation of lipids leads to rancidity, change in food quality, such as color, aroma, flavor, texture and even the nutritive value of the food. Controlling and monitoring of lipid oxidation during meat processing or storage are important for precooked convenient meat products for home, fast food, and institutional uses.<sup>1</sup>

In order to protect lipids, avoid deterioration of appearance, and microbial growth in meat product manufacturers, several food additives with antimicrobial and antioxidant properties were used.<sup>2–4</sup> The use of food additive in food industry to preserve flavor or enhance its taste and appearance improves food processing, preservation, quality and safety, as well as increased production, cost-effectiveness and sustainability.

Nowadays, there is an increased demand for healthier food products without chemical preservatives, resulting in a need to avoid the use of synthetic additives. This has favored the use of natural additives or alternative methods to extend shelf life and/or improve safety. Chitosan is one of these alternatives. Chitosan has been used in food products as preservative in fresh pork sausages,<sup>5,6</sup> fresh pork burgers,<sup>7</sup> preservative for meat and

meat products,<sup>4,8</sup> frozen beef burgers,<sup>2</sup> and edible coatings for fruit and vegetables.<sup>9,10</sup>

Chitosan is a cationic polysaccharide made from alkaline *N*-deacetylation of chitin. It has attracted attention as a biomedical material, owing to its unique biological activities including anti-tumor activities,<sup>11</sup> immunostimulating effects,<sup>12</sup> cholesterol-lowering effects,<sup>13</sup> antimicrobial effects,<sup>14</sup> antioxidant activity,<sup>15–19</sup> wound healing effects,<sup>20</sup> free radical scavenging activities,<sup>21</sup> chelating activity that selectively binds protein and metals,<sup>22</sup> and as potential food preservative of natural origin due to its antibacterial and antifungal activities.<sup>23</sup> The applications of chitosan in food industry and medicine is limited because of its high molecular weight resulting in its low solubility in aqueous media.<sup>24</sup> It is important to improve the water solubility of chitosan to expand its usefulness in the food industry. The solubility and functionality of chitosan can be improved by introducing active groups onto the hydroxyl of carbon 3 and 6 and/or amino groups carbon 2.<sup>25</sup> In recent years, radiation degradation of chitosan to low molecular weight chitosan or oligochitosan is more interesting to develop many successful applications; some of them commercialized for use in agriculture, health care, food, and environmental protection.

Antioxidants are an important group of food additives as health protecting factors for prevention of oxidative damage and

extend shelf life of foods. Recently, the antioxidant activity of chitosan derivatives has attracted attention by various investigators.<sup>14,18,19</sup> There has been increasing interest in finding natural antioxidants. Since they can protect the human body from free radicals and retard the progress of many chronic diseases. Ascorbic acid is one form of vitamin C (Vitamin C, VC) and is a naturally occurring organic compound with antioxidant properties and can be used as antioxidant and food additives due to the presence of the enediol moiety. The biochemical functions of VC such as scavenger reactive oxygen species, antiviral and antitumor are of increasing interests.<sup>26</sup>

In this study, CS was exposed to  $\gamma$  rays at different doses to prepare low MW followed by incorporation with VC to obtain water soluble CSVC complexes. The antioxidant activity of CSVC complexes and their effectiveness in reducing the lipid peroxidation of meat during refrigerated storage will be examined.

## MATERIALS AND METHODS

### Materials

Chitosan (CS), Aldrich, high molecular weight (MW)  $1.9 \times 10^6$  Da, the degree of deacetylation (DD)  $\geq 85\%$ . Pure ascorbic acid (Vitamin C, VC), El-Nasr pharmaceutical chemical company. 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) was purchased from Sigma Chemicals. Ferric chloride (97%) and ferrous chloride (98%) were supplied from BDH chemicals, India. Potassium ferricyanide was supplied from Riedel laboratory reagents. Trichloroacetic acid and 2-thiobarbituric acid (TBA, 98%) were supplied from SUVCHEM laboratory chemicals, India. Other reagents and solvents such as acetic acid and ethanol were of analytical grade.

### Analytical Methods

UV absorbance was measured by a UV spectrophotometer JASCO V-560, in the range of 190–400 nm. For UV measurements, diluted solutions of CS (0.1 wt % in 1% acetic acid) and CSVC aqueous solutions (0.1 wt % in dist. Water) were prepared.

The transmittance (%) was carried out using infrared spectrophotometer FT-IR 6300 JASCO, Japan, in the form of KBr pellets. Solid CS or CSVC complexes were grinded with KBr pellets and the then compressed for a disk.

The molecular weight (MW) of CSVC was determined by gel permeation chromatography (GPC) 1100 Agilent instrument equipped with aqueous column GPC-SEC start up kits with a flow rate of 1 mL/min. The MW was obtained by using a calibration curve of polyethylene oxide standards of known MWs.

The mean particle size and surface distribution were observed by Transmission Electron Microscopy (TEM; JEOL JEM-100CX, Shimadzu, Japan, with an acceleration voltage of 80 kV). For TEM observations, the samples were prepared by making a suspension from CSVC complex solution in distilled water using ultrasonic water bath. The suspension was centrifuged to separate the polymer matrix and collimate the large size particles. Then a drop of the suspension was put into the carbon grid and left to dry at room temperature.

### Radiation Degradation of CS

According to our previous reported method,<sup>27</sup> CS was irradiated by  $\gamma$  rays at different doses of 25, 50, and 100 kGy by  $^{60}\text{Co}$   $\gamma$

rays in solid form at dose rate of 3.52 kGy/h to prepare different MWs of CS and named as CS25, CS50, and CS100.

### Synthesis of CSVC Complexes

Water soluble CSVC complexes were synthesized through the ionic interaction reaction between the amine groups of glucosamine units of different MW of CS backbone and VC. Briefly, certain amount of unirradiated CS (CS0) or CS25 or CS50 or CS100 was dissolved in 100 mL containing 1% acetic acid then VC solution (mol/mol; the concentration of VC is equal to the concentration of glucosamine unit of CS) was added dropwise into CS solutions with stirring for 4 h. The product was precipitated by acetone, filtered, washed with acetone again to remove unreacted compounds, and then dried to obtain different types of water soluble CSVC complexes according to the value of CS irradiated dose namely CS0VC, CS25VC, CS50VC, and CS100VC.

### Determination of Scavenging Activity (%)

Measurement of scavenging activity on DPPH radicals was determined according to the method described previously.<sup>28</sup> The utilized solvent for DPPH is ethanol and for CSVC complexes is water. Briefly, a reaction mixture containing 1.5 mL of DPPH solution (0.1 mM, in 95% ethanol) was incubated with 1.0 mL of different concentrations of CSVC complex solutions (10–100  $\mu\text{g/mL}$ ). The reaction mixture was shaken well and incubated for 15 min at room temperature and the absorbance of the resulting solution was measured at 517 nm. Ascorbic acid was used as positive control under the same assay conditions. Negative control was without any CS or CSVC. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity was calculated from the decrease in absorbance value at 517 nm in comparison with negative control. Experiment was done in triplicates. The scavenging activity (%) was calculated using the following equation:

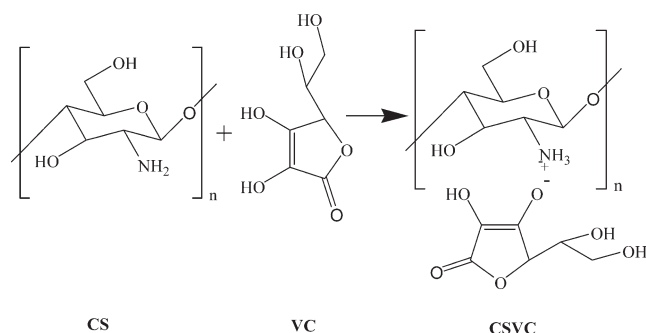
$$\text{Scavenging activity (\%)} = [(A_{\text{control } 517 \text{ nm}} - A_{\text{samples } 517 \text{ nm}}) / A_{\text{control } 517 \text{ nm}}] \times 100$$

### Determination of Reducing Power

The reducing power was determined by the method described previously.<sup>29</sup> Briefly, a reaction mixture containing 1.0 mL of different concentrations of CSVC solution (0.1–2 mg/mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated for 20 min at 50°C in waterbath. To stop the reaction, 2.5 mL of 10% trichloroacetic acid was added to the reaction mixture, followed by centrifugation for 10 min at speed of 1500 rpm. Then, 2.5 mL supernatant was mixed with 2.5 mL distilled water, and 0.5 mL ferric chloride solution (0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as positive control under the same assay conditions. Negative control was without any CS or CSVC. Increasing the absorbance of the reaction mixture indicates the increase in reducing power of the samples.

### Preparation of Dipping Solutions for Meat Treatment

Diluted solutions of CS0, VC, CS0VC, CS25VC, CS50VC, and CS100VC were prepared at different concentrations of 0.05, 0.1, and 0.2% and stirred for 1–2 h before using for dipping of meat.



**Scheme 1.** Formation of CSVC complex.

### Treatment of Meat with CSVC Complexes and Storage Conditions

Fresh minced meat was obtained from a local market. 10 g of minced meat sample were dipped on the prepared solutions for 10 min. Samples without any dipping treatment was used as control. The treated meats were then gently drained on a tissue paper, placed in polyethylene bags and stored in the refrigerator at 4°C for 7, 15, and 21 days. Each treatment contained three replicates and the experiment was repeated three times.

### Assessment of Lipid Oxidation of Meat

Lipid oxidation was assessed according to the thiobarbituric acid reactive substance (TBARS) assay<sup>30</sup> accordingly to the concentration of malondialdehyde (MDA) formed during 0, 7, 15, and 21 days of refrigerated storage (4 ± 1°C). Malondialdehyde (MDA) is one of the aldehydes formed during lipid oxidation in the meat. Triplicate 10 g samples (for each group) were homogenized with 50 mL of 7.5% trichloroacetic acid (TCA) solution. The homogenate was filtered and 5.0 mL aliquot was transferred to a clean screw capped tube and mixed with 5.0 mL of freshly prepared 0.02 M TBA solution. The mixture was put in a boiling water bath for 35 min until color formation, and then it was left to cool until submitted to conventional spectrophotometry. The TBARS values obtained by measuring the absorbance of the developed pink color were measured at wavelength 532 nm. TBARS values were expressed as equivalent of mg MDA/kg meat.

The decrease (%) in MDA formed at storage time (day) = [(TBARS value of treated samples - TBARS value of control) / TBARS value of control] × 100

### Data Analysis

An average value of the replicate analyses was used in calculations of sample variation and significance testing. All statistical analysis was performed with SPSS (SPSS). Values are presented as means. LSD test is basically a set of individual t tests. LSD calculates the smallest significant between two means as if a test had been run on those two means. This enables to make direct comparisons between two means from two individual groups.

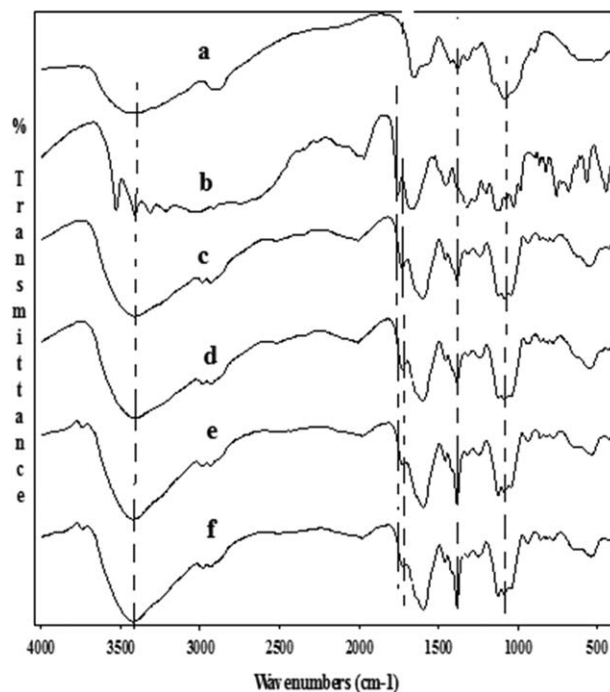
## RESULTS AND DISCUSSION

### Synthesis and Characterization of CSVC Complexes

VC presents several electrophilic groups and contains four hydroxyl groups in positions 2, 3, 5, and 6 with different acidities allowing acid–base reactions. The –OH in position 3 is the

more acidic one ( $pK_a = 4.2$ ), while the hydroxyl in position 2 has a  $pK_a$  of 11.6, and those in positions 5 and 6 behave as secondary and primary alcohol ( $pK_a \approx 17$  and 16), respectively.<sup>31</sup> The acidic hydroxyl in position 3 of VC was expected to react with the amino group of CS, converting it into ammonium ions. The result CSVC complex has the properties of both components in addition can improve the solubility of CS. The possible reaction mechanism was shown in Scheme 1.

FT-IR spectra of CS (Figure 1, curve a) shows basic characteristic absorption bands at 3440  $\text{cm}^{-1}$  (O–H and N–H stretch), 1651  $\text{cm}^{-1}$  corresponding to the stretching of amide C=O, 1598  $\text{cm}^{-1}$  (N–H bend), 1387  $\text{cm}^{-1}$  (amide), 1154  $\text{cm}^{-1}$  (asymmetric bridge-O-stretch) and 1089  $\text{cm}^{-1}$  (skeletal vibration involving the C–O stretch). FT-IR of irradiated CS was reported in our previous paper,<sup>15</sup> it was found there is no significant change between FT-IR of unirradiated and irradiated CS indicating that the stability of the  $\beta$ -glycosidic bonds and distribution of glycosidic bonds in the molecular chains of CS. FT-IR spectra of VC (Figure 1, curve b) shows four peaks at 3527, 3413, 3317, and 3217  $\text{cm}^{-1}$  were attributed to the four –OH groups at C(6)–OH, C(3)–OH, C(5)–OH, and C(2)–OH, respectively. The bands at 1754 and 1673  $\text{cm}^{-1}$  are corresponding to lactone C=O and Cdbond|C stretching, respectively. The C–H stretching is assigned at 3029–2920  $\text{cm}^{-1}$ .<sup>32</sup> The bands at 1386 and 1260  $\text{cm}^{-1}$  are due to C–O stretching vibration. FT-IR spectrum of CS0VC (Figure 1, curve c) shows that the bands at 3440 and 1598  $\text{cm}^{-1}$  characteristic of  $\text{NH}_2$  bending vibrations gradually weakened and a new absorption band appeared at 1759  $\text{cm}^{-1}$  due to C=O group of ascorbic acid. The peak at 1759  $\text{cm}^{-1}$ , which was the stretch vibration of lactone C=O group forming intramolecular



**Figure 1.** FT-IR spectra of (a) CS, (b) VC, (c) CS0VC, (d) CS25VC, (e) CS50VC, and (f) CS100VC.

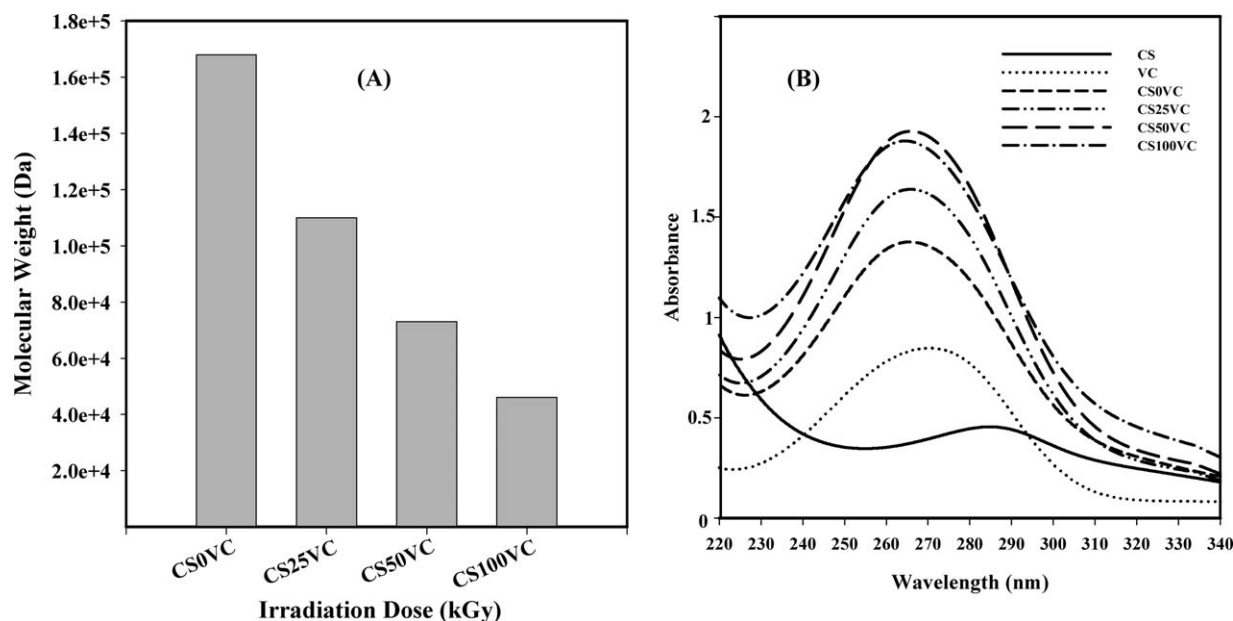


Figure 2. (A) The MW of CSVC complexes and (B) UV-Vis spectra of CS, VC, and CSVC complexes.

H-bond in VC, was shifted to  $1729\text{ cm}^{-1}$  at a reduced intensity indicating the formation of complex between CS and VC. These results suggest that the  $\text{NH}_2$  groups in the CS chains were protonated by acetic acid and the  $\text{H}^+$  supplied by ascorbic acid.<sup>33</sup> The FT-IR spectra of CS25VC, CS50VC, and CS100VC (Figure 1, curves d–f) show a decrease in peak intensity at  $3440\text{ cm}^{-1}$  indicated the reduction of free  $\text{—NH}_2$  groups after the formation of CSVC mixtures. This is due to radiation degradation of CS lead to decrease inter- and/or intramolecular hydrogen bonding between the  $\text{—OH}$  and  $\text{—NH}_2$  groups and to increase the amount of functional groups formed such as  $\text{—OH}$  groups and so the intensity of the band at  $3340\text{ cm}^{-1}$  corresponding to  $\text{—OH}$  and  $\text{—NH}_2$  groups. The vibrational band at  $1100\text{ cm}^{-1}$  that corresponds to the ether bond in the pyranose ring has no significant change during the reaction with VC, which indicates that, the stability of the  $\beta$ -glycosidic bonds and distribution of glycosidic bonds in the molecular chains of CS.

Figure 2(A) shows the MW of CSVC decreases with decreasing the starting Mw of CS. The MW of CS at 0, 25, 50, and 100 kGy was  $1.9 \times 10^6$ ,  $7.8 \times 10^5$ ,  $3.1 \times 10^5$ , and  $9.7 \times 10^4$  Da, respectively. The MW of the obtained CSVC mixtures after treatment with VC were  $1.68 \times 10^5$ ,  $1.1 \times 10^5$ ,  $7.3 \times 10^4$ , and  $4.6 \times 10^4$  Da, respectively. It was found that the MW of CSVC mixtures are even lower than original CS because ascorbic acid (a natural organic acid) can act as a solvent for CS dissolution which affects some degradation of CS and the size of molecules in the solution becomes smaller, causing reduction in MW of the obtained CSVC complexes.

Figure 2(B) shows UV spectra of VC exhibits a strong absorption band at about 265 nm which is due to the  $\pi\text{—}\pi^*$  excitation of the  $\text{C}(2)=\text{C}(3)$  double bond. UV spectra of CSVC complexes showed a strong absorption band at 265 nm and the peak intensity increased with decreasing the MW of starting CS as a result of the effect of irradiation dose on degradation of starting

CS. The peak intensity of irradiated CS affects the peak intensity of CSVC complexes after reaction with VC. In a previous reported paper,<sup>27</sup> the effect of irradiation dose on degradation of CS was studied, it was found that MW decrease with increasing the irradiation dose. Also, from UV spectra of CS shows absorption band around 280–315 nm and the peak intensity increased with increasing the irradiation dose. This may be caused by the  $n \rightarrow \sigma^*$  transition for the amino groups of CS and may also assigned to the  $n \rightarrow \pi^*$  transition for the carbonyl groups.

Figure 3 shows the morphology of (a) CS50VC and (b) CS100VC mixtures analyzed by TEM. The CSVC mixtures show a dark, solid and consistent structure resulted in aggregated with diameters ranged from 23.2 to 82 nm. These CSVC complexes were formed by ionic interaction between positively charged amino groups of CS and negatively charged of acidic hydroxyl group at C3 of VC results in a matrix structure with solid and consistent spherical shapes. CS50VC complex possessed different diameters of 82, 52.6, 46.5, 40, and 23.2 nm. Also, CS100VC complex possessed different diameters of 55.4, 48.3, 42.1, 41.1, 37.2, 31.5, and 28.2 nm. The size of CS100VC is more uniform than that of CS50VC.

#### Scavenging Activity (%)

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological system.<sup>34</sup> DPPH radical has been used to test the ability of compounds to act as free radical scavengers and thus to evaluate the antioxidant activity.<sup>8</sup> DPPH has a characteristic absorption band at  $\lambda_{\text{max}}$  517 nm, which decreases significantly on exposure to proton radical scavengers.<sup>35</sup> Figure 4 shows the scavenging activity (%) of unirradiated CS, irradiated CS, VC and different types of CSVC complexes on DPPH radicals. Generally, the scavenging activity (%) on DPPH increases with increasing the concentration. The scavenging activity (%)

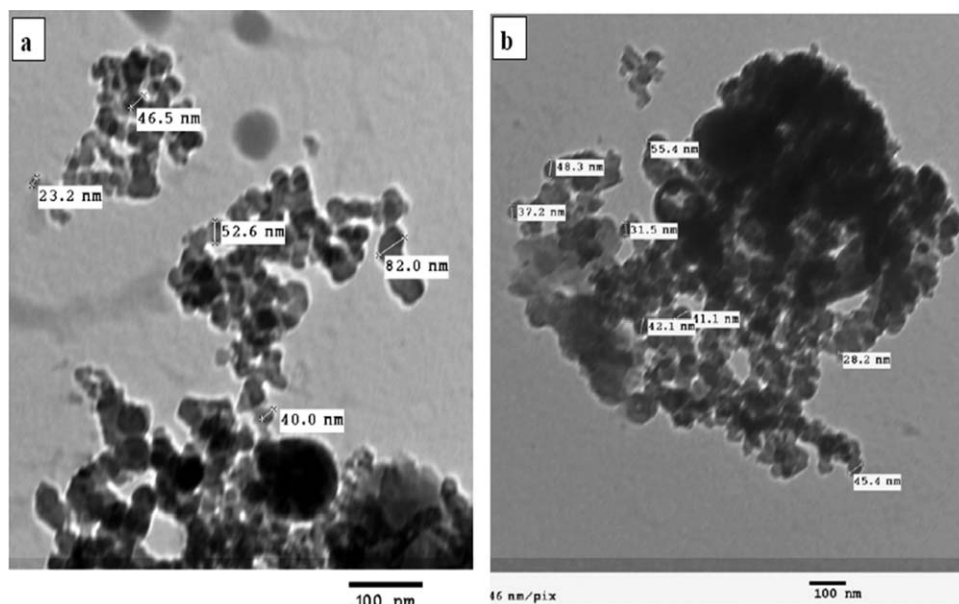


Figure 3. TEM images of (a) CS50VC and (b) CS100VC.

of CS was enhanced by  $\gamma$ -irradiation. The lower Mw of CS, the higher scavenging activity. At 70  $\mu\text{g}/\text{mL}$  concentration, the scavenging activity (%) of CS irradiated at 0, 25, 50, and 100 kGy was 3, 14.5, 19.8, and 28.5%, respectively if compared with that of VC (42%). This is due to high Mw of CS which has compact structure, thus making the overall effect of their intramolecular hydrogen bonds stronger lead to decrease the reactivity of hydroxyl and amino groups. On the contrary, low Mw CS has a less compact structure, thus making the overall effect of intra-

molecular hydrogen bonding less effective and so increase the reactivity of hydroxyl and amino groups.

Incorporation of CS molecules with VC had a synergistic effect on increasing the scavenging activity (%) on DPPH rather than their individual effects. The CSVC complexes with lower Mw of CS have a promising effect on increasing the scavenging activity. The CS100VC had the highest scavenging activity on DPPH. At the concentration 70  $\mu\text{g}/\text{mL}$ , the scavenging activity (%) on

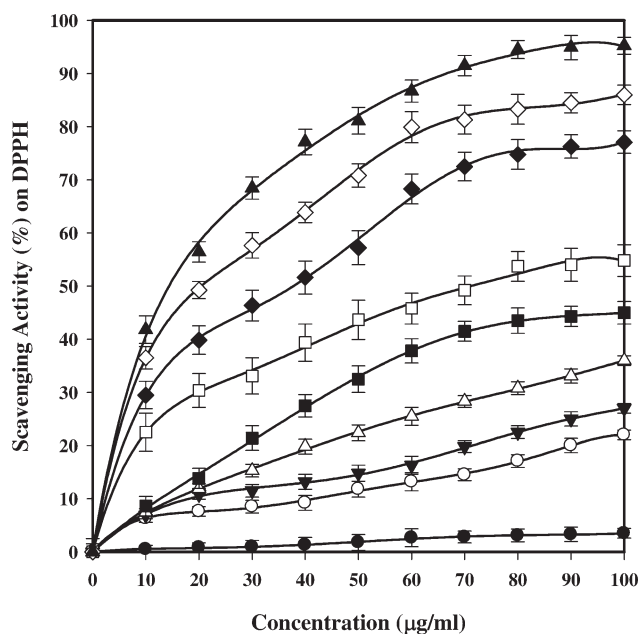


Figure 4. Scavenging activity (%) on DPPH radicals of (●) CS, (○) CS25; (▼) CS50; (△) CS100 (■) VC; (□) CS0VC; (◆) CS25VC; (◇) CS50VC, and (▲) CS100VC. Each value is expressed as mean.

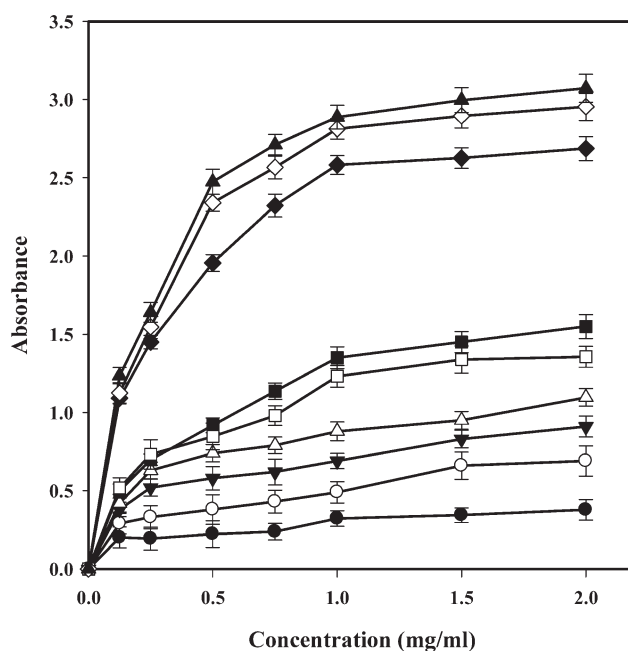


Figure 5. Reducing power of (●) CS, (○) CS25; (▼) CS50; (△) CS100 (■) VC; (□) CS0VC; (◆) CS25VC; (◇) CS50VC, and (▲) CS100VC. Each value is expressed as mean.

**Table I.** Values of TBARS Value of Meat During 7, 15, and 21 Days of Storage at  $4 \pm 1^\circ\text{C}$  Using Different Concentration of Different Treatments of CS, VC, CS0VC, CS25VC, CS50VC, and CS100VC

Storage time	7 days			15 days			21 days		
Concentration	0.05%	0.1%	0.2%	0.05%	0.1%	0.2%	0.05%	0.1%	0.2%
Control	0.5042a			0.7481a			0.955a		
CS	0.4888b	0.4884b	0.466b	0.6953b	0.6494b	0.6354b	0.8613b	0.851b	0.8442b
VC	0.416c	0.4006c	0.405c	0.6162c	0.6003c	0.5902c	0.73c	0.7205c	0.6823bc
CS0VC	0.3782d	0.3544d	0.3374d	0.5623d	0.5494d	0.5315cd	0.6182d	0.5838d	0.5671cd
CS25VC	0.3396e	0.3051e	0.2957e	0.5157e	0.5015e	0.4957de	0.6059e	0.5808e	0.525d
CS50VC	0.2913f	0.2248f	0.2147f	0.4911f	0.4832f	0.4692f	0.5776f	0.5752f	0.4955d
CS100VC	0.2233g	0.1663g	0.1259g	0.4567g	0.4249g	0.4036f	0.566g	0.5247g	0.4761d
LSD	0.0058	0.0015	0.0055	0.0015	0.0038	0.0039	0.0013	0.0015	0.138

Each value is expressed mean.

Means with same letters in a column are not significantly different ( $P < 0.05$ ).

DPPH of CS0VC, CS25VC, CS50VC, and CS100VC was 49.2, 72.5, 81.3, and 91.5, respectively. Percentage of inhibition  $\text{IC}_{50}\%$  is used very frequently as parameters characterizing the antioxidant power.  $\text{IC}_{50}$  of CS25VC, CS50VC, and CS100VC was 32, 22.5, and 18  $\mu\text{g}/\text{mL}$ , respectively. These results revealed that the prepared CSVC mixtures have a good antioxidant activity.

#### Reducing Power

Antioxidant activity has been reported to be concomitant with reducing power. Figure 5 shows the reducing power of unirradiated CS, irradiated CS, VC, and different types of CSVC complexes. CSVC complexes with low Mw CS showed high reducing power and the reducing power increases with increasing the concentration. The reducing power reach its maximum value at 1.0  $\text{mg}/\text{mL}$  concentration then leveled off with further increase in concentration. At 1.0  $\text{mg}/\text{mL}$  concentration, the reducing power of CS0, CS25, CS50, CS100, VC, CS0VC, CS25VC, CS50VC, and CS100VC was 0.3225, 0.495, 0.69, 0.8809, 1.3503, 1.2314, 2.582, 2.813, and 2.8877, respectively. Increasing the absorbance indicates increasing reducing power activity. CS50VC and CS100VC complexes showed high reducing properties compared to CS or VC. The increase in reducing power of CSVC complexes indicates the enhancement of their antioxidant activity suggesting their possible use as antioxidants for preventing flavor changes caused by lipid peroxidation.

#### Lipid Oxidation of Meat During Storage

During storage of meat, lipid peroxides are formed with a subsequent formation of peroxy radicals, followed by a decomposition phase to yield aldehydes such as malondialdehyde (MDA).<sup>36</sup> MDA is an equivalency for stating TBARS values as  $\text{mg MDA}/\text{kg}$  of meat. Table I presented TBARS values for treated and untreated meat.

In the present study, control samples (untreated) had TBARS values of 0.5042, 0.7481, and 0.955  $\text{mg MDA}/\text{kg}$  at 7, 15, and 21 days of storage respectively, and would therefore be perceived as rancid already after the 15 days of storage. Whereas TBARS values for treated samples were decreased until the end of the storage period. TBARS values changes accordingly to storage period, the type and concentration of CSVC complex. The addi-

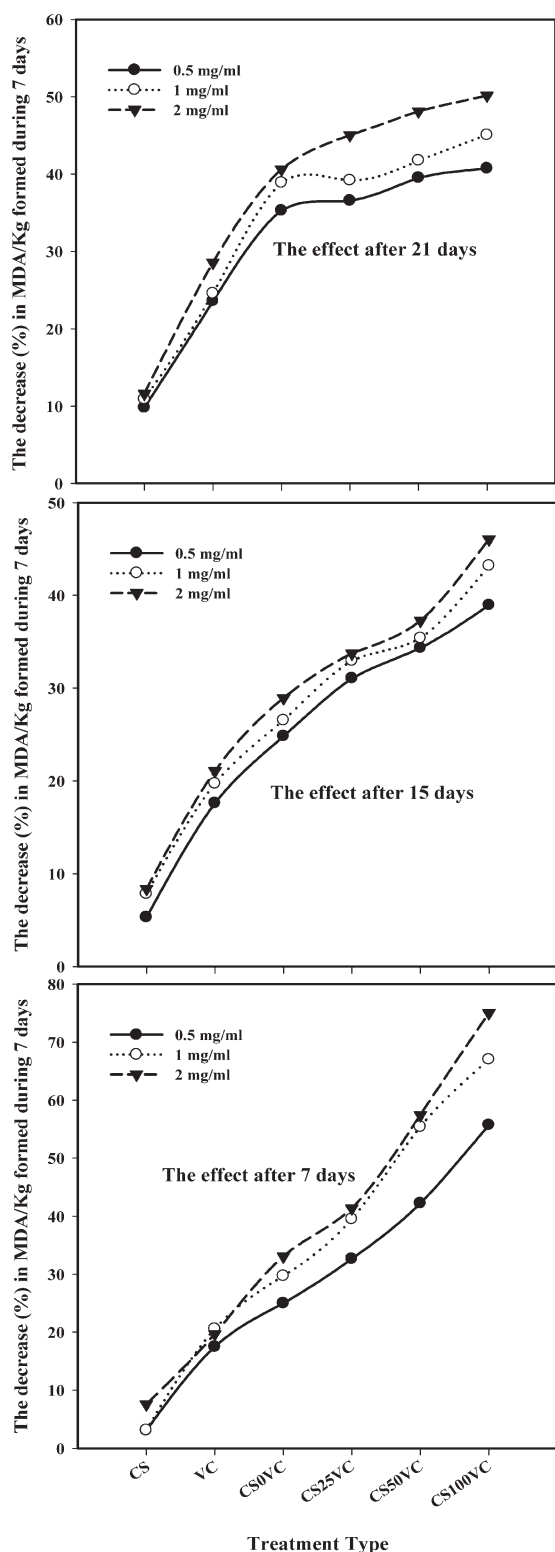
tion of CSVC complexes to meat during storage lead to decrease TBARS values indicating the decrease in rate of lipid oxidation of meat. Using 0.2% concentration of CS or VC treated samples; the TBARS values reached 0.8442 or 0.6823  $\text{mg MDA}/\text{kg}$  meat at 21 days and, respectively. Meanwhile, using CS0VC complex the TBARS values was 0.5671  $\text{mg MDA}/\text{kg}$  meat with a decrease about 32.8–16.8% in the formation of MDA which improves the protection of meat samples against lipid oxidation.

In comparison the effect of treatment of CSVC complexes with the control and those treated by CS or VC as shown in Table I, in general the TBARS values of CS25VC, CS50VC, and CS100VC showed lower values with increasing the concentration of treated CSVC complexes and storage period and so great effect on delaying peroxidation of meat by extending the induction period as results of their good antioxidant properties. However, the TBARS value of the CS100VC treated group is lower than the CS25VC and CS50VC treated samples due to the effect of decreasing the MW of CS incorporated with VC.

Figure 6 shows the decrease (%) in  $\text{MDA}/\text{kg}$  meat as result of TBARS values during 21 days of storage at  $4 \pm 1^\circ\text{C}$  using different concentration and different treatments. The results revealed that CS50VC and CS100VC complexes showed a higher effect on decreasing lipid peroxidation of meat. Also, the decrease (%) in MDA formed was affected by the concentration, type of the treatment and storage time.

Using CS100VC complex at 0.05% concentration and storage time of 7, 15, and 21 days, the decrease (%) in  $\text{MDA}/\text{kg}$  of meat was 55, 39, and 40.7%, respectively. While, using 0.1% concentration the decrease (%) in  $\text{MDA}/\text{kg}$  was 67, 43, and 45%, respectively, and using 0.2% concentration the decrease (%) was 75, 46, and 50%, respectively.

Compared to CS and VC results, after storage time of 7, 15 and 21 days, the decrease (%) in  $\text{MDA}/\text{kg}$  of meat at 0.05% concentration was 3, 5.3, and 9.8%, respectively for using CS and 17.5, 21, and 23.5% for using VC. While at 0.2% concentration the decrease (%) was 7.5, 8.3, and 11.6%, for using CS and 19.6, 21, 28.5%, respectively for using VC. The combinations of VC with lower MW CS (obtained by  $\gamma$  rays) improve the solubility



**Figure 6.** The decrease (%) in MDA formed during 7, 15, and 21 days of storage of meat at  $4 \pm 1^\circ\text{C}$  using 0.05, 0.1, and 0.2% concentration of different treatment from CS, VC, CS0VC, CS25VC, CS50VC, and CS100VC.

of CS and enhanced their activities. CSVC complex showed highly antioxidant effect suggesting their possible use for delaying lipid peroxidation.

## CONCLUSION

CSVC complexes were synthesized in the range of nanoparticles through incorporation of VC with CS molecules of different MW prepared by exposure to  $\gamma$  rays. CSVC complexes had a synergistic effect on increasing the scavenging activity (%) on DPPH radicals and high reducing power rather than their individual effects. The treatment of meat by CSVC complexes showed a highly significant decrease in TBARS values. CS50VC and CS100VC complexes showed high decrease (%) in MDA formed during storage of meat. CS100VC complex could decrease about 75% of MDA formed after 7 days of storage. It can be concluded that CSVC complexes could be used as antioxidants for lipid storage.

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