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Effect of γ-rays on carboxymethyl chitosan for use as antioxidant and preservative coating for peach fruit

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ABSTRACT

Carboxymethyl chitosan (CMCS) was synthesized by alkylation of chitosan using monochloroacetic acid and characterized by FTIR and ^{1}H-NMR spectroscopies. Different molecular weights (Mws) of CMCS were prepared by radiation degradation of CMCS in the solution form at different irradiation doses. The structural changes and Mw of degraded CMCS were confirmed by UV–Vis, FTIR and GPC. The antioxidant activity of CMCS was evaluated using scavenging effect on DPPH radicals, reducing power and ferrous ion chelating activity assays. The antioxidant activity of CMCS enhanced with decreasing CMCS Mw. The possible practical use of CMCS as preservative coating for peach fruit by dipping treatment after 10 days of storage at ambient temperature was investigated. The CMCS with lower Mw had a good effect on delaying spoilage and decreasing malondialdehyde (MDA) content of peach fruits suggesting their possible use as antioxidant and preservative coating.

1. Introduction

Edible coating materials such as polysaccharides, proteins, essential oils may serve as edible coatings for extending the shelf life of post-harvested fruits and vegetables (Bosquez-Molina, Ronquillo-de Jesús, Bautista-Banos, Verde-Calvo, & Morales-Lopez, 2010; Rojas-Graü, Tapia, & Martín-Belloso, 2008).

Extending the shelf life of fresh peach using edible coating is beneficial to preserve and to maintain the freshness of peach due to short postharvest life at room temperature and high susceptibility to pathogens causing brown which is a major disease on peach fruit (Sasaki, Cerqueira, Sestari, & Kluge, 2010; Zhou, Schneider, & Li, 2008).

Chitosan and its derivatives like oligochitosan, have been reported to control postharvest diseases effectively. Chitosan is safe, nontoxic, biocompatible, and biodegradable natural alkaline polysaccharide derived from the deacetylation of chitin (Carlson, Taffs, Davison, & Stewart, 2008). Chitosan has been widely applied in medicine, biotechnology, water treatment, agriculture, and food science (Kumar, 2000). In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coating (Devlieghere, Vermeulen, & Debevere, 2004), protect plants against microorganisms (Pospieszny, Chirkov, & Atabekov, 1991). Chitosan has been successfully used in many post harvested fruits and vegetables, such as grape, strawberries, berry, jujube and fresh cut lotus root through single coating or comprehensive treatments (Vu, Hollingswort, Leroux, Salmieri, & Lacroix, 2011; Wang & Gao, 2013; Xing et al., 2010). Chitosan can form a film on fruit and vegetable surfaces and reduces respiration rate by adjusting the permeability of carbon dioxide and oxygen. The $-NH_2$ group of chitosan may also restrain the propagation of harmful germs, thus effectively controlling fruit decay (Devlieghere et al., 2004). Chitosan and oligochitosan contributed positively on senescence resistance induction of peach fruit against brown rot caused by *Monilinia fructicola* and delayed fruit softening (Ma, Yang, Yan, Kennedy, & Meng, 2013).

In an attempt to improve the water solubility and to enlarge the applications of chitosan, many chemical modifications made to introduce hydrophilic groups to prepare chitosan derivatives with good water solubility, biocompatibility and unique bioactivities were carried out by acylation reaction (Vanichvattanadecha et al., 2010), Maillard reaction (Ying, Xiong, Wang, Sun, & Liu, 2011), quaternary reaction (Verheul et al., 2008), carboxymethyl reaction (Sreedhar, Aparna, Sairam, & Hebalkar, 2007), and alkylation reaction (Chung, Tsai, & Li, 2006). A variety of techniques used to prepare oligochitosan including acid hydrolysis (Jeon & Kim, 2000), ultraviolet degradation (Wang, Huang, & Wang, 2005), gamma radiation processing (Kang, Dai, Zhang, & Chen, 2007; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005), and oxidative

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degradation with hydrogen peroxide or ammonium persulfate in presence of gamma radiation (El-Sawy, Abd El-Rehim, Elbarbary, & Hegazy, 2010). Oligochitosan exhibits a wide variety of activities, including plant growth promotion (Abd El-Rehim et al., 2011; Aftab et al., 2011; Chmielewski et al., 2007; El-Sawy et al., 2010; Khan, Khan, Aftab, Idrees, & Naeem, 2011), antitumor activities (Suzuki et al., 1986), antiviral activity (Pospieszny et al., 1991), antimicrobial activity (Park, Je, Byun, Moon, & Kim, 2004), fat lowering and hypocholesteromic effects (Czechowska-Biskup, Rokita, Ulanski, & Rosiak, 2005), immuno-stimulting properties (Matsuo & Miyazono, 1993), free radical scavenging activities (Anraku et al., 2008) and as antioxidant agent for food preservation (Chen, Liau, & Tsai, 1998; Rao, Chander, & Sharma, 2005).

In recent years, great interest in finding natural antioxidants from plant materials to be used in foods or medicinal materials. Antioxidants are compounds capable of delaying, retarding or preventing autooxidation processes caused by reactive oxygen (Shahidi, Janitha, & Wanasundara, 1992). Antioxidants are also widely used as additives in fats and oils and in food processing to prevent or delay spoilage of foods. In addition of defense response, antioxidant was also associated closely with fruit resistance against disease.

In the present study, carboxymethyl chitosan (CMCS) will be prepared followed by γ-rays treatment to prepare different Mws of CMCS. The antioxidant activity of the prepared CMCS will be studied. The possible use of CMCS as antioxidant and preservative coating by dipping treatment for peach fruit at ambient temperature will be investigated.

2. Experimental

2.1. Materials and methods

Chitosan, DD 85%, Mw 420 kDa, Aldrich. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (Ferrozine) were purchased from Sigma Chemicals. EDTA, ferric chloride and ferrous chloride were supplied from BDH. Potassium ferricyanide was supplied from Riedel laboratory reagents. Thiobarbituric acid, monochloroacetic acid and trichloroacetic acid were supplied from SUVCHEM laboratory chemicals. Other reagents and solvents were of analytical grade.

2.2. Preparation of CMCS

The carboxymethylation process of CS into water soluble CMCS was carried out according to the method reported previously (Chen et al., 2004; Qian, Zhou, Ma, Wang, & He, 1996). Briefly, CS powder (10 g) was suspended in 100 ml of isopropyl alcohol and the resulting slurry was stirred in a 500 ml flask at room temperature. 25 ml of 10 N aqueous NaOH solution, divided into five equal portions, was then added to the stirred slurry over a period of 25 min. The alkaline slurry was stirred for additional 30 min. Subsequently, monochloroacetic acid (60 g) was added, in five equal portions, at 1 min intervals. Heat was then applied to bring the reaction mixture to a temperature of 60 °C and stirring at this temperature was continued for 3 h. Afterward, the reaction mixture was filtered and the filtered solid product (CMCS) was thoroughly rinsed with methanol. The resultant CMCS was dried in an oven at 60 °C. The Mw of the produced CMCS was determined by GPC technique. In addition, the degree of substitution (DS) of prepared CMCS was estimated using potentiometric titration against 0.1 M aqueous NaOH with aid of the following equation (El-Sherbiny, 2009):

$$\mathrm{DS} = \frac{161 \times A}{m - 58 \times A} \quad \text{where } A = V \times C$$

where m is the mass (g) of CMCS, V and C are the volume and molarity of NaOH solution, respectively. The values 58 and 161 represent the molecular weights of the carboxymethyl group and the glucosamine unit (chitosan skeleton unit) of CS, respectively.

2.3. Irradiation

CMCS solutions (1%) were irradiated by ^{60}Co γ-rays at different doses of 10, 20 and 30 kGy was at dose rate of 2.58 kGy/h.

2.4. Preparation of coating solutions

CMCS (unirradiated and irradiated) aqueous solutions were prepared in distilled water at a concentration of 1 mg/ml and stirring for 1–2 h.

2.5. Coating and storage conditions of peach fruits

Selected peach (*Prunus persica* (L.) Batsch) fruit samples were distributed randomly and divided into five different treatments as followed: (a) control (untreated), (b) peach treated by unirradiated CMCS 0 kGy, (c) peach treated by irradiated CMCS at 10 kGy, (d) peach treated by irradiated CMCS at 20 kGy and (e) peach treated by irradiated CMCS at 30 kGy. All the treatments were performed in triplicate. The peach fruits were dipped into the prepared solutions for 5 min then stored at room temperature. Water was used for the immersion of control samples. Each treatment contained three replicates and the experiment was repeated three times.

2.6. Characterization methods

The transmittance was measured using infra-red spectrophotometer JASCO FTIR 6300, Japan in the form of KBr pellets in the range of 400–4000 cm^{-1}. UV absorbance was measured by UV–vis spectrophotometer Jasco V-560, Japan, in the range from 190 to 900 nm. The MWs of the unirradiated and irradiated CMCS were determined by Gel permeation chromatography (GPC) 1100 Agilent, USA.

2.7. Determination of antioxidant activity

2.7.1. Determination of scavenging activity (%) on DPPH radicals

Measurement of free radical scavenging activity on DPPH radicals was determined according to the method described previously (Yamaguchi, Takamura, Matoba, & Terao, 1998). Briefly, 1.5 ml of DPPH solution (0.1 mM, in 95% ethanol) was incubated with different concentrations of unirradiated and irradiated CMCS solutions. The reaction mixture was shaken well and incubated for 15 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank (control). Ascorbic acid was used for comparison as antioxidant materials. The radical scavenging effect was measured as a decrease in the absorbance of DPPH and can be calculated using the following equation:

$$\text{Scavenging activity } (\%) = \left[1 - \left(\frac{A_{\text{samples } 517\,\text{nm}}}{A_{\text{control } 517\,\text{nm}}}\right)\right] \times 100$$

2.7.2. Determination of reducing power

Different concentrations of unirradiated and irradiated CMCS solutions (1 ml) were mixed with 0.2 M sodium phosphate buffer pH 6.6 (2.5 ml) and 1% (w/v) potassium ferricyanide (2.5 ml). The mixtures were incubated for 20 min at 50 °C. The reaction was terminated by adding 10% (w/v) trichloroacetic acid (2.5 ml) to the mixtures, followed by centrifugation for

10 min at 1500 rpm. 2.5 ml supernatant was mixed with 2.5 ml distilled water and 0.5 ml ferric chloride (0.1%, w/v) solution and the absorbance was measured at 700 nm (Yen & Duh, 1993). Increasing the absorbance of the reaction mixture indicates the increase in reducing power of the samples. Ascorbic acid was used for comparison as antioxidant materials.

2.7.3. Determination of chelating activity

A 0.25 ml of unirradiated and irradiated CMCS solutions of different concentrations with 0.5 ml ferrous chloride (2 mM) and 0.25 ml Ferrozine (5 mM) shaken well, and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against blank (Dinis, Madeira, & Almeida, 1994). EDTA was used for comparison as antioxidant materials. The chelating ability of all samples to chelate ferrous ion was calculated using the following equation:

$$\text{Chelating activity}\ (\%) = \left[1 - \left(\frac{A_{\text{sample}\ \ 562\,\text{nm}}}{A_{\text{control}\ \ 562\,\text{nm}}}\right)\right] \times 100$$

2.7.4. Assay of malondialdehyde (MDA) content

MDA content was measured according to the previously reported method (Xing, Wang, Feng, & Tan, 2008). 3 g peach fruit from each treated group were homogenized with 15 ml of 10% trichloroacetic acid and centrifuged at 15,000 rpm for 20 min. One milliliter of supernatant was mixed with 3 ml of 0.5% 2-thiobarbituric acid, heated at 95 °C for 20 min, and then immediately cooled in an ice water bath. The absorbance was measured at 532 and 600 nm after centrifugation at 3000 rpm for 10 min and the value for non-specific absorbance 600 nm was subtracted. MDA concentration was calculated by an extinction coefficient of 155 $Mm^{-1}\,cm^{-1}$ through the formula:

$$(\text{MDA}\ \ \mu\text{mol/g}\ \ \text{fresh}\ \ \text{weight}) = \frac{(OD_{532} - OD_{600}) \times 40}{0.155 \times \text{formula weight}}.$$

2.8. Antimicrobial activity

The antimicrobial activity of different Mws of CMCS was evaluated by applying the agar plate diffusion technique. unirradiated CMCS and irradiated CMCS were screened in vitro for their antibactericidal activity (against Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli*) and antifungal activity (against *Aspergillas flavus* and *Candida albicans*). In this method, a standard 5 mm diameter sterilized filter paper disk impregnated with samples (1 mg/ml of DMF) was placed on an agar plate seeded with the test organism. The agar plates were then incubated for 24 h at 37 °C for bacteria and 28 °C for fungi. After incubation, the interrupted growth zone (zone of inhibition) around the test material was measured (mm/mg) and used as quantitative indicator of antibacterial and antifungal effectiveness of CMCS. The values obtained were the average of 5 measurements on the same plate at different zones.

2.9. Data analysis

All statistical analysis was performed with SPSS (SPSS Inc., Chicago, IL, USA). Differences at $\rho < 0.05$ were considered to indicate statistical significance.

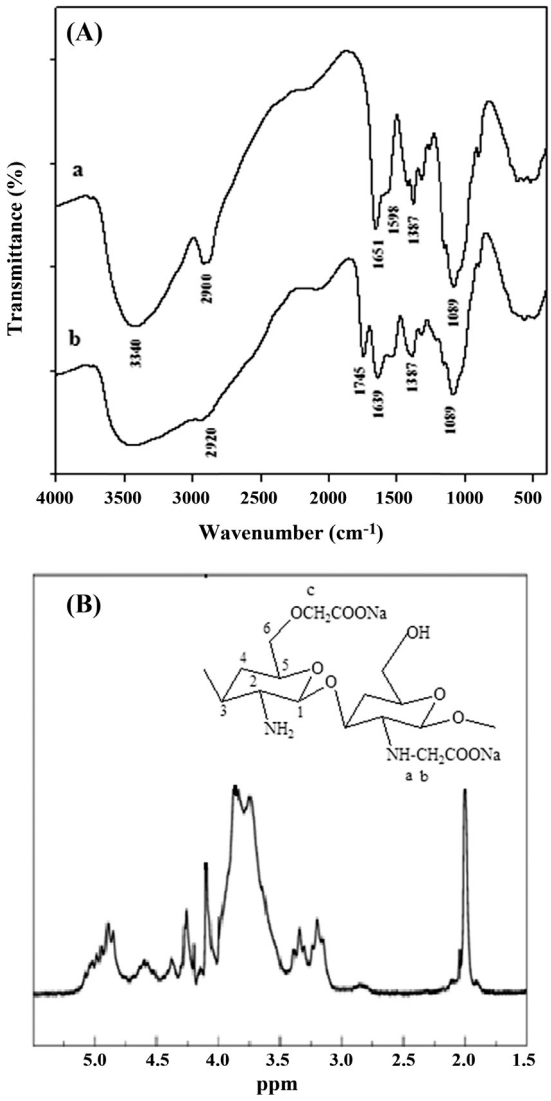

Fig. 1. (A) FT-IR spectra of (a) CS and (b) CMCS; (B) ^{1}H NMR spectrum of CMCS.

3. Results and discussion

3.1. Synthesis and characterization of water soluble CMCS

CMCS is one of the most important kinds of chitosan derivatives. CMCS is nontoxic in vitro and in vivo (Kennedy, Costain, McAlister, & Lee, 1996). In this study, a water soluble CMCS was synthesized by the direct alkylation method using monochloroacetic acid. The Mw and degree of substitution of CMCS were determined as followed 460 kDa and 0.68, respectively. Fig. 1A shows FTIR spectra of CS and its modified CMCS. FTIR spectrum of CS (Fig. 1A, curve a) shows basic characteristic absorption bands at 3440 cm^{-1} (O—H and N—H stretch), 1651 cm^{-1} corresponding to the stretching of C=O group of amide, 1598 cm^{-1} (N—H bend), 1387 cm^{-1} (Amide), 1154 cm^{-1} (asymmetric bridge-O-stretch) and 1089 cm^{-1} (skeletal vibration involving the C—O stretch). For CMCS, the spectrum is different from the spectrum of CS. The FTIR spectrum of CMCS (Fig. 1A, curve b) shows new absorption band at 1745 cm^{-1} due to —COO^- group. The absorption band appears at 1651 cm^{-1} was shifted to 1639 cm^{-1} due to carboxymethylation of CS. Compared with the bands of CS, the bands at 1650, 1387 and 1089 cm^{-1} became more intense in the CMCS spectrum indicating carboxymethylation on both the amino and hydroxyl groups of CS (Chen & Park, 2003).

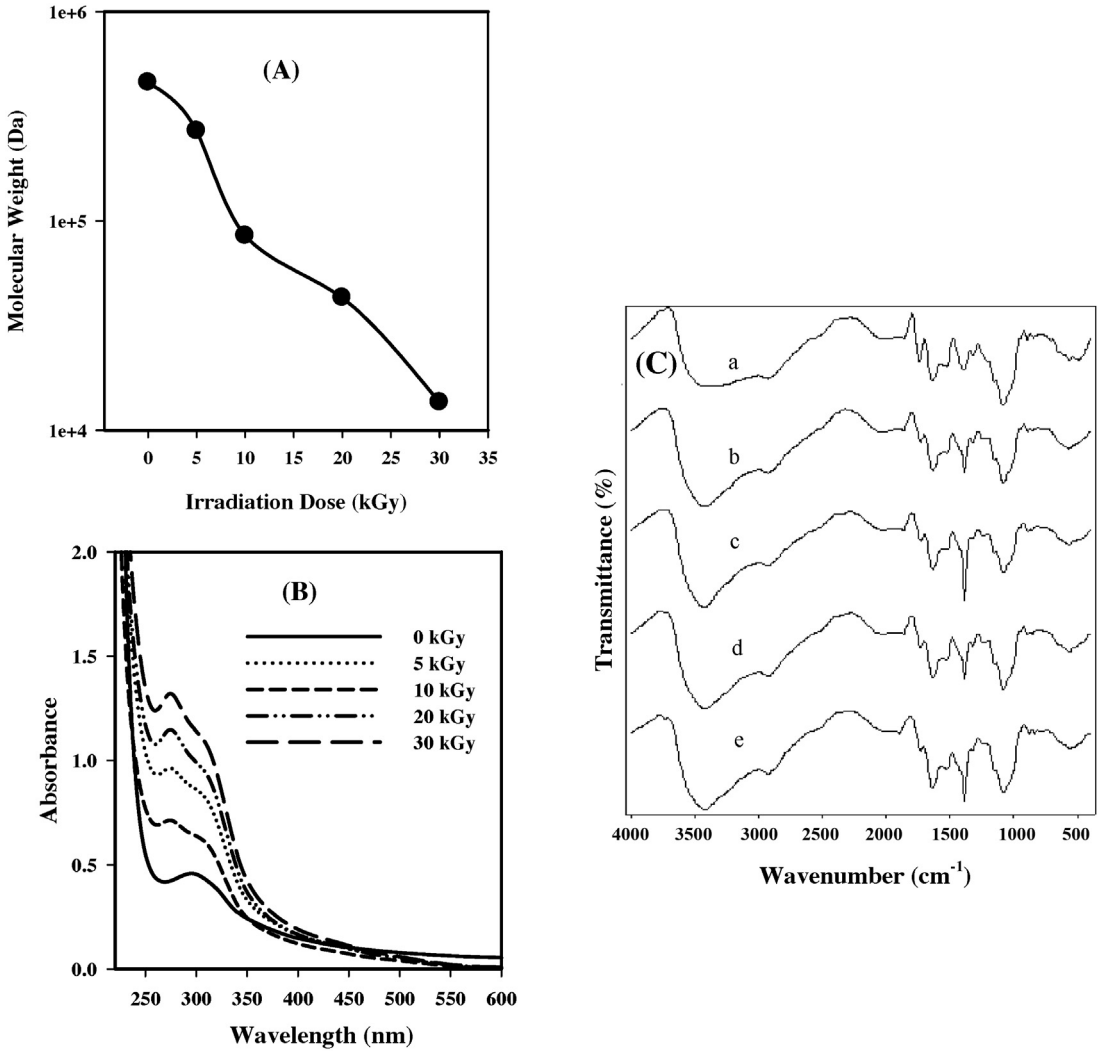

Fig. 2. The structural changes of CMCS after γ-irradiation degradation, (A) the Mw measured by GPC; (B) UV–vis spectra of irradiated CMCS and (C) FT-IR spectra of (a) unirradiated CMCS, (b) irradiated CMCS at 5kGy, (c) irradiated CMCS at 10 kGy, (d) irradiated CMCS at 20 kGy, and (e) irradiated CMCS at 30 kGy.

Fig. 1B shows the ^{1}H NMR of CMCS as reported (Mourya, Inamdar, & Tiwar, 2010) shows a characteristic peaks at $\delta = 4.58$, 2.66, 3.54, 3.72, 3.59 and 3.74 ppm were attributed to the H-1, H-2, H-3, H-4, H-5 and H-6 respectively. The peak at $\delta = 2.37$ ppm was attributed to the methylene proton (CH_2) of carboxymetgyl group. The peaks at $\delta = 2.12$, 4.14 and 3.23 ppm were attributed to the H-a, H-b and H-c respectively.

3.2. Radiation synthesis and characterization of CMCS with different Mws

The structural changes of CMCS with different Mws were confirmed using GPC, UV–vis and FTIR spectroscopies. Fig. 2A shows the change in the Mw of CMCS after irradiation at different doses. It was found that there is a remarkable reduction in Mw of CMCS with increasing the irradiation dose. The Mw of CMCS decreased from 460 kDa to 13.6 kDa using 30 kGy irradiation dose.

Radiation yield of degradation (Gd) is usually used to evaluate the radiation susceptibility of a polymer, which was determined by using Alexander–Charlesby–Ross equation (Charlesby, 1960):

$$\frac{1}{M_{nD}} - \frac{1}{M_{n0}} = G(d) \times 1.04 \times 10^{-7} \times D$$

where D is the absorbed dose in kGy and M_{nD} and M_{n0} are the number average molecular weights of the CMCS before and after irradiation. It was found that the radiation yield of degradation (Gd) for CMCS irradiated at 5, 10, 20 and 30 kGy was 2.94, 9.22, 10.13 and 22.87 respectively.

Fig. 2B shows UV–vis spectra of unirradiated and irradiated CMCS. The unirradiated CMCS showed broad absorption bands around 298 nm. The irradiated CMCS had two absorption peaks at 275 and 309 nm and the intensity of these peaks increased with increasing the irradiation dose. The observed two peaks of the irradiated chitosan may be due to the presence of carbonyl and carboxyl groups. The obtained results are consistent with those reported before (Nagasawa, Mitomo, Yoshii, & Kume, 2000; Ulanski & Rosiak, 1992). The aqueous solution of CMCS was pall yellowish color that changed to dark yellowish color by radiation confirmed the formation of the unsaturated bonds. As the irradiation dose increases, the yellowish color intensity increases to deep ones.

Fig. 2C shows FTIR spectra of irradiated CMCS. In Fig. 2C, curves b–e exhibited most of the characteristic absorption peaks of native CMCS. The absorption peaks appeared at 1089 and 3420 cm^{-1} corresponding to ether bond and (O—H and N—H stretch) became stronger. The fact should be related to the scission of glycosidic bonds leads to the formation of some hydroxyl group, which is manifested as an increase in their intensity at 3420 cm^{-1} due to

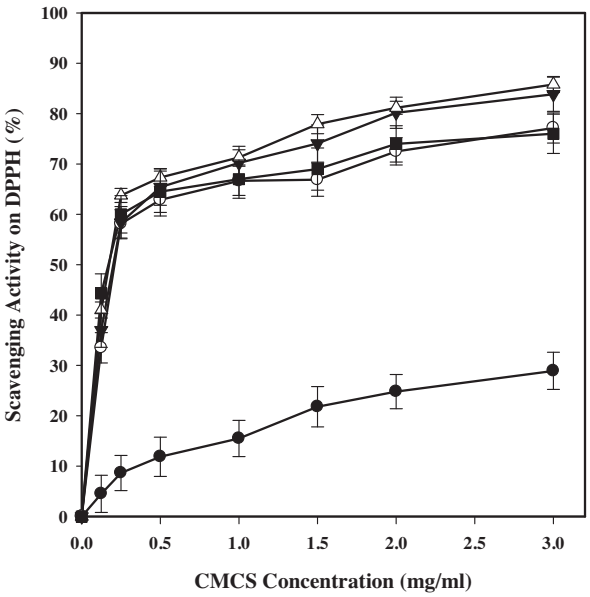

Fig. 3. Scavenging activity (%) on DPPH radicals of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) ascorbic acid as a reference. Each value is expressed as mean.

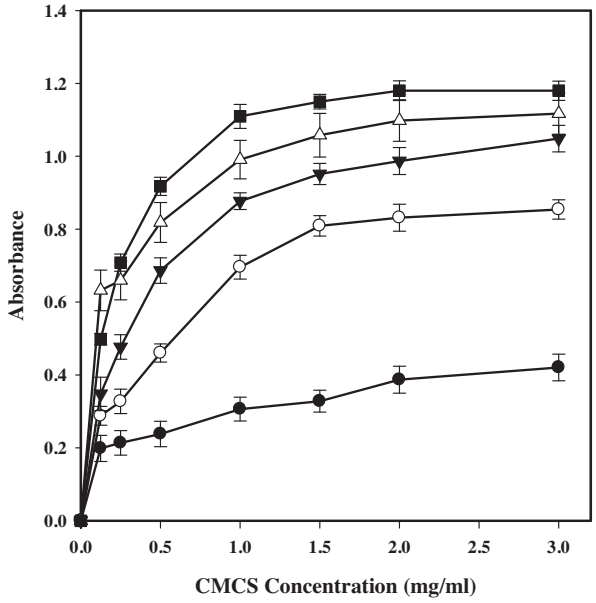

Fig. 4. Reducing power of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) ascorbic acid as a reference. Each value is expressed as mean.

the decrease of intermolecular hydrogen bonding. The vibrational band at 1080–1100 cm^{-1} that corresponds to the ether bond in the pyranose ring has no significant change, which indicates that, the stability of the β-glycosidic bonds and distribution of glycosidic bonds in the molecular chains of CMCS and the main polysaccharide chain structure was almost remained during degradation process.

3.3. Determination of scavenging effect (%) on DPPH radicals

The DPPH radical is a stable organic free radical acting as an electron acceptor from antioxidants with a characteristic absorption at λ_{max} 517 nm, which decreases significantly on exposure to proton radical scavengers (Curcio et al., 2009). It is a useful reagent for evaluation of antioxidant activity of compounds. This assay is based on the principle that DPPH• can accept a hydrogen (H) atom from the scavenger molecule (antioxidant), resulting into reduction of DPPH• (diphenylpicrylhydrazyl radical) to DPPH-H (diphenylpicrylhydrazine; nonradical), the purple color changes to yellow with concomitant decrease in absorbance at 517 nm.

Fig. 3 shows the scavenging effect of unirradiated and irradiated CMCS on DPPH radicals. The scavenging activity (%) on DPPH increases as the concentration of the CMCS increases. Also, the irradiated CMCS shows an increase in scavenging activity (%) on DPPH radicals compared with unirradiated ones. As the Mw of CMCS decreases, the scavenging activity (%) increases. It was found that the irradiation of CMCS at low dose (10 kGy) increases the scavenging activity (%) on DPPH radicals 3 times. At 1 mg/ml concentration, the scavenging activity (%) on DPPH radicals for unirradiated and irradiated CMCS at 10, 20 and 30 kGy was 15.5, 67, 70.2 and 71.3%, respectively. The irradiation of CMCS at 10 kGy gives enough degradation to increase radical scavenging effect as a result of a change in Mw comparable to that ascorbic acid.

Percentage of inhibition IC_{50} (the concentration of antioxidant which provides 50% inhibition) are used very frequently as parameters characterizing the antioxidant power. The IC_{50} (%) on DPPH radicals for irradiated CMCS at 10 kGy was about 0.174 mg/ml. The results revealed that irradiated CMCS has a good antioxidant activity.

In our previous study (Abd El-Rehim, El-Sawy, Hegazy, Soliman, & Elbarbary, 2012), the scavenging activity (%) on DPPH radicals for CS at 1 mg/ml concentration was 8% but the chemical modification of CS to CMCS increases the scavenging effect to 15.5%. This is due to CMCS has a significant characteristic of its solubility in water. Also, the scavenging activity (%) on DPPH radicals for CS irradiated at 30 kGy was 65% but only irradiation of CMCS at 10 kGy scavenging activity (%) was 67%. The results revealed that the chemical modification of CS to water soluble one is well done to reduce the dose and cost required for such technologies. It was reported (Sun, Yao, Zhou, & Mao, 2008) that IC_{50} of N-carboxymethyl chitosan oligosaccharides on DPPH radicals was 0.71 mg/ml. Meanwhile, in the present study, the IC_{50} (%) for CMCS irradiated at 10 kGy was 0.174 mg/ml.

3.4. Reducing power

Reducing power assay has also been used to evaluate the ability of natural antioxidants to donate electrons (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Sun, Zhu, Xie, & Yin, 2011). The reducing power of different Mws of CMCS was determined by the potassium ferricyanide reduction method by measuring the absorbance at 700 nm. Stronger absorbance indicates increased reducing power. In the assay, a potential antioxidant will reduce the ferric ion in Fe^{3+}/ferricyanide complex to the ferrous ion (Fe^{2+}) and the yellow color of the test solution changes to various shades of green color depending upon the reducing power of antioxidant. This is due to the reduction of the Fe^{3+}/ferricyanide complex to the ferrous Fe^{2+} form.

Fig. 4 shows the reducing power of irradiated CMCS. The results showed that the lower Mw of CMCS exhibited high reducing power, and the reducing power increased with increasing of CMCS concentration. At 2 mg/ml concentration, the reducing power of unirradiated CMCS was 0.38. Meanwhile, the reducing power of irradiated CMCS at 10, 20 and 30 kGy became 0.83, 0.98 and 1.1, respectively. The introduction of electron donating carboxymethyl group enhanced the electron cloud density of active hydroxyl and amino groups, thus the electron donating activity increased and the reducing power improved.

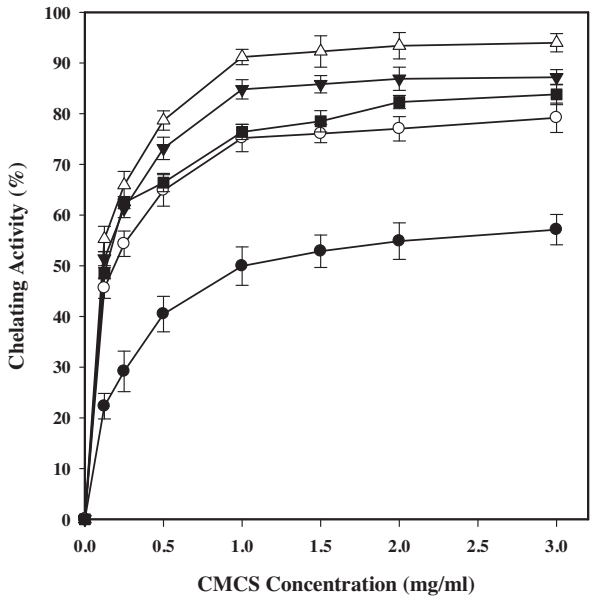

Fig. 5. Chelating activity (%) of (●) unirradiated CMCS, (○) irradiated CMCS at 10 kGy, (▼) irradiated CMCS at 20 kGy, (△) irradiated CMCS at 30 kGy and (■) EDTA as a reference. Each value is expressed as mean.

3.5. Chelating activity (%)

Ferrous ions are the most effective prooxidants in the food system (Yamaguchi, Tatsumi, Kato, & Yoshimitsu, 1988), and can stimulate lipid peroxidation and start a chain reaction, which lead to the deterioration of flavor and taste in food (Gordon, 1990). The high ferrous ion chelating activities would be beneficial if they were formulated into foods. Fig. 5 shows chelating activity (%) of unirradiated and irradiated CMCS with different concentration toward the ferrous ion. The chelating activity (%) increased with increasing CMCS concentration and by γ-rays treatment of CMCS. With increasing the irradiation dose, the Mw of CMCS decreases, the higher the ferrous ion chelating activity obtained. This is due to the free functional groups formed after radiation induced degradation of CMCS such as OH groups help to form CMCS-Fe^{2+} complexes. Indeed, nitrogen atoms in chitosan hold free electron doublets that can react with metal cations and uptake metal cations by a chelation mechanism.

At 0.5 mg/ml concentration, the chelating activity (%) of unirradiated CMCS was 40.4%. While, the chelating activity (%) of CMCS irradiated at 10, 20 and 30 kGy was 64.8, 73.1 and 78.6%, respectively. Also, at 2 mg/ml concentration, the chelating activity (%) became 54.8, 77, 86.8 and 93.4%, respectively. The $IC_{50\%}$ of the chelating activity (%) of CMCS irradiated at 10 kGy, CMCS irradiated at 20 kGy, CMCS irradiated at 30 kGy and EDTA was 0.154, 0.115, 0.107 and 0.103, respectively.

3.6. Antibacterial and antifungal activities of CMCS

Table 1 shows antibacterial and antifungal activities as a function of exposure of the Gram positive *S. aureus* (bacteria), the Gram negative *E. coli* (bacteria), *A. flavus* (funus) and *C. albicans* as (fungus) to unirradiated and irradiated CMCS, which caused a decrease in viable cell counts. It was observed that the irradiated CMCS was effective in decreasing the viable cell count of *S. aureus and E. coli* (inhibition zone diameter 13–14 mm/sample). It was found that the antibacterial and antifungal activities affected by the Mw of CMCS. The irradiation of CMCS at 10 kGy is enough to see the effect of irradiation on the antibacterial and antifungal efficiency. The inhibitory effects differed with regard to the Mw of CMCS and the type of bacterium. CMCS irradiated at 10 kGy was the highest in antibacterial and antifungal efficiency. The inhibitory index against *E. coli*, *S. aureus*, *A. flavus* and *C. albicans* was 41, 50, 82 and 71%, respectively compared with the standard materials. CMCS irradiated at 10 kGy had higher antifungal activity more than antibacterial activity.

The positively charged nature of CMCS molecules enhances their antibacterial activity and facilitates their binding with bacterial cell wall and leads to the inhibition of bacterial cell growth forming polyelectrolyte complexes (Choi et al., 2001; Kim, Lee, Lee, & Park, 2003). This could act as impermeable layer around the cell and suppress the metabolic activity of the bacteria by blocking of nutrient permeation through the cell wall.

The use of antioxidant materials for food preservation has been mentioned. If such materials also possess antibacterial properties to kill invading bacteria, the preservation process can be achieved. Furthermore, in food packaging application, materials with both antibacterial and antioxidant properties can inhibit bacterial growth as well as scavenge oxygen species to prevent food from oxidative spoilage. With such dual functionalities in the packaging materials, an extended shelf life of the food can be realized (Srinivasa & Tharanathan, 2007).

3.7. Applicability of CMCS as preservative coating for peach fruit

Application of irradiated CMCS has been investigated as preservative coating of peach fruit by dipping treatment after 0, 3, 7 and 10 days of storage as shown in Fig. 6. The coating of peach with irradiated CMCS, especially at 20 and 30 kGy has prolonged the storage life from 3 to 7 days at ambient temperature keeping peach with good color without spoilage. While, brown rot was observed for untreated peach (control) and the brown rot increased with increasing the storage time led to rancidity of peach and spoiled completely. This effect is due to the antifungal activity of CMCS enhanced by irradiation. CMCS has been shown to have beneficial effects in delaying ripening of whole fruits when used soon after harvest (Meheriuk & Lau, 1998).

During storage of peach fruit, reactive oxygen species (ROS) can cause peroxidation and form toxic products such as malondialdehyde (MDA) as an indicator to assess the progress of fruit damage (Liu et al., 2012). Fig. 7 shows the effects of unirradiated

Table 1
Antibacterial and antifungul activity of unirradiated CMCS and irradiated CMCS at different doses. Each value is expressed as mean. Means with same letters in a column are not significantly different.

Sample	Inhibition zone diameter (mm/mg sample)			
	E. coli (G−)	*S. aureus* (G+)	*Aspergillas flavus*	*Candida albicans*
Control	0	0	0	0
Tetracycline (antibacterial agent)	31	28	–	–
Amphotericin B (antifungal Agent)	–	–	17	21
Unirradiated CMCS	9^a	9^a	0	0
CMCS at 10 kGy	13^b	14^b	14^b	15^b
CMCS at 20 kGy	13^c	13^c	12^c	13^c
CMCS at 30 kGy	12^d	13^c	11^d	12^c

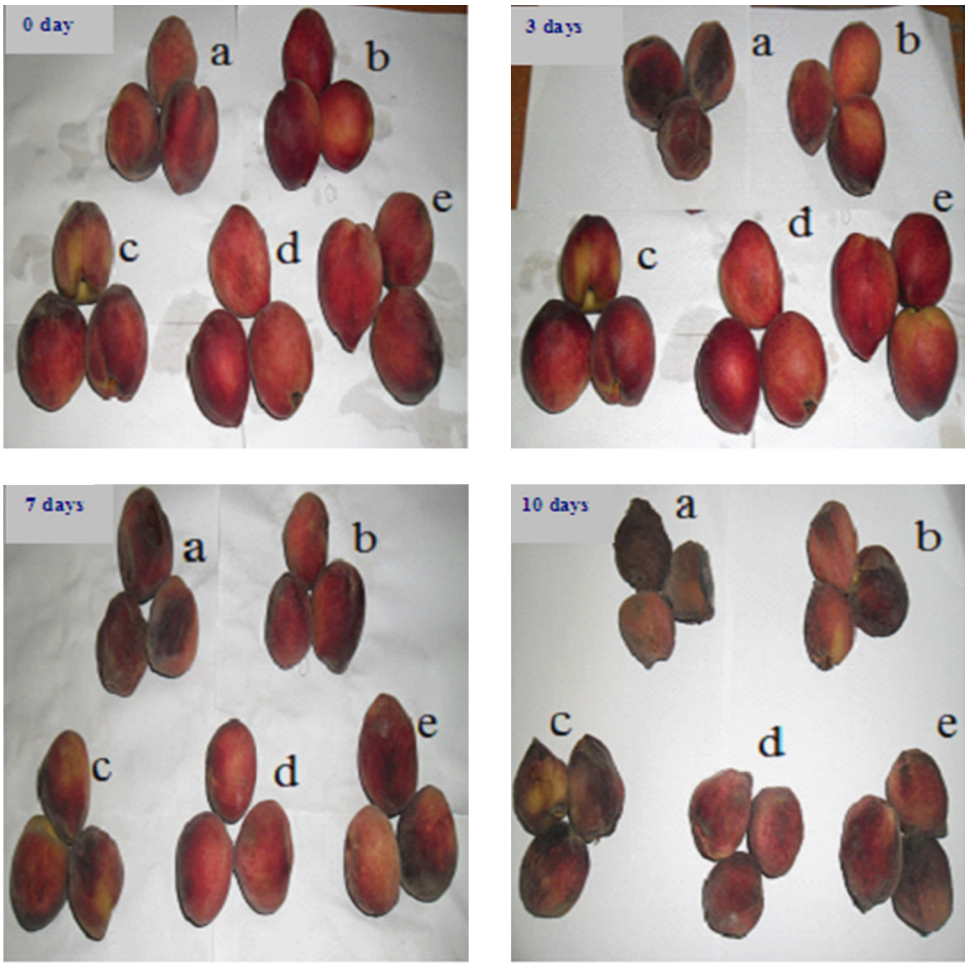

Fig. 6. Appearance of peach fruits treated by dipping with unirradiated and irradiated CMCS solution (1 mg/ml) after 0, 3, 7 and 10 days of storage. (a) Control (untreated peach), (b) peach treated with unirradiated CMCS, (C) peach treated with CMCS irradiated at 10 kGy, (d) peach treated with CMCS irradiated at 20 kGy, and (e) peach treated with CMCS irradiated at 30 kGy.

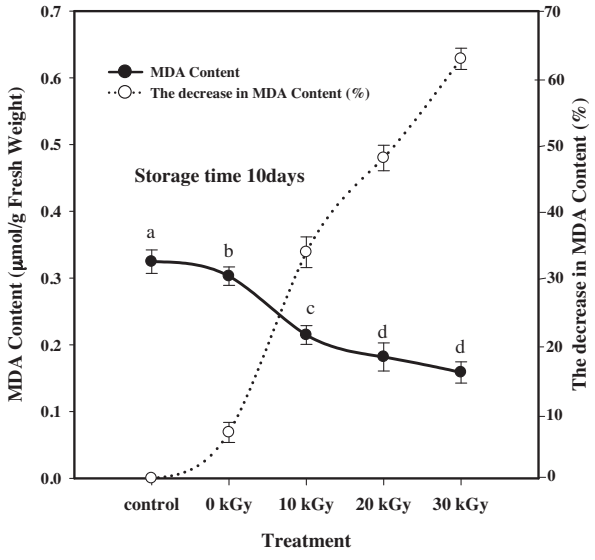

Fig. 7. Effect of unirradiated and irradiated CMCS on MDA content (μmol/g fresh weight) of peach fruits and the decrease (%) in MDA during 10 days of storage, compared with untreated samples (control). Each value is expressed as mean. Means with same letters are not significantly different.

and irradiated CMCS on the MDA content (μmol/g fresh weight) of peach fruits and the decrease (%) in MDA content during 10 days of storage, compared with untreated samples (control). It was found that the treatment of peach with irradiated CMCS at 10, 20 and 30 kGy has MDA content 0.22, 0.18 and 0.15 (μmol/g fresh weight) with decrease (%) of 33.8, 48 and 62.8 (%), respectively lower than untreated and unirradiated CMCS peach 0.32 and 0.3 (μmol/g fresh weight). These results suggest the treatment of peach fruit with irradiated CMCS was better than that of control and unirradiated CMCS and more effective in controlling brown rot and delaying senescence caused by artificial inoculation in peach fruit during 10 days of storage at ambient temperature.

The application of chitosan based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries was favorable in extending shelf life, maintaining quality and controlling decay of strawberries (Wang & Gao, 2013). The effects of chitosan and oligochitosan on resistance induction of peach fruit against brown rot caused by *M. fructicola* showed significant effect on controlling this disease. Moreover, chitosan and oligochitosan delayed fruit softening and senescence (Ma et al., 2013). The application of chitosan coating on quality and shelf life of peeled litchi fruit showed significant results on maintaining quality attributes and extending shelf life of the peeled fruit (Dong, Cheng, Tan, Zheng, & Jiang, 2004).

4. Conclusion

Carboxymethylation of chitosan followed by γ-rays treatment to lower Mw improves their antioxidant activity. The irradiated CMCS had higher scavenging activity (%) on DPPH radicals. The irradiation of CMCS at 10 kGy showed enough degradation to increase the antioxidant, antibacterial and antifungal efficiency than unirradiated CMCS against both bacteria and fungi. The coating of peach fruits with irradiated CMCS by dipping treatment has promising effect on prolonging the storage life with good color without spoilage, controlling brown rot and decreasing MDA content. The use of irradiated CMCS will be a promising alternative as antioxidant and preservative coating for peach fruits.

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