

Comparative Study on the Antioxidative Properties of Some Natural Polymers Degraded by γ -rays

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RADIATION induced degradation of chitosan, Na-alginate and carrageenan was carried out to prepare oligosaccharides with different molecular wt. Structural and average molecular wt changes of such oligosaccharides were determined by gel permeation chromatography (GPC), Fourier transform infra-red (FT-IR) and ultraviolet (UV-Vis.) spectroscopy. FT-IR and UV-Vis. studies revealed that during radiation degradation process, the main polysaccharide chain structure was almost remained. Comparative study on the antioxidative properties of chitosan, Na-alginate and carrageenan oligosaccharides of different molecular wt was investigated. Radical mediated lipid peroxidation inhibition, scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, reducing power and the ferrous ion chelating activity assays were used to evaluate the antioxidant activity. The irradiation process enhances the antioxidant activity of such polysaccharides. The lower the molecular wt of oligosaccharides is the higher the antioxidant activity. The antioxidant activity of irradiated chitosan was higher than Na-alginate and carrageenan. At 30 kGy, the inhibition concentration (IC₅₀) on DPPH radicals was 0.154, 0.359, 0.438 and 0.140 mg/ml for chitosan, Na-alginate carrageenan, and ascorbic acid, respectively.

Keywords: γ -rays, natural polymers, degradation, chitosan, oligosaccharides, antioxidant activity.

The demands on new polymeric materials are increasing rapidly with the development of biotechnological science. Chitosan, Na-alginate and carrageenan are biodegradable natural polymers. They have wide range of applications such as wound healing and food packaging etc. (Carlson *et al.*, 2008).

They received much attention as biomaterials for value-added products, especially in food industry (Ikeda 2003), as a growth promoter or protector for some plants in the agricultural applications (El-Sawy *et al.*, 2010) and as antioxidant agents for food preservation (Shahidi *et al.*, 1999).

Antioxidants are classified as compounds capable of delaying, retarding or preventing auto-oxidation processes caused by reactive oxygen and they act as oxygen scavengers, react with free radicals and chelate catalytic metals and thus retard oxidative deterioration (Kanat *et al.*, 1998). In recent years, great interest in finding natural antioxidants from plant materials or from different sources has been drawn more and more attention for use in foods or medicinal materials to replace synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene. A multitude of natural antioxidants have already been isolated from different kinds of plant materials (Ramarathnam *et al.*, 1995). Also, seaweed is considered to be a rich source of antioxidants (Cahyana *et al.*, 1992). In recent years, polysaccharides from different sources were reported to be useful candidates in the search for an effective, nontoxic substance and have been demonstrated to play an important role as free radical scavengers *in vitro* and antioxidants for the prevention of oxidative damage in living organisms (Zhang *et al.*, 2004). In many of antioxidants applications, specific molecular wt of polysaccharides are required. Radiation processing of natural polymers is an area of current research for development of new applications. Irradiation of these polymers led to the reduction of molecular wt by scission of glycosidic linkage (Charlesby 1981). Controlling the degree of degradation, uniform molecular wt distribution, saving achieved in the chemicals (used in conventional methods) on a cost basis, and environmentally friendly process are the beneficial effects of using radiation technology in these industries.

In the present work, preparation of low molecular wt oligosaccharides from chitosan, Na-alginate and carrageenan using ionizing radiation was carried out to increase their antioxidant activities.

Materials and Methods

Chitosan, degree of deacetylation 85% of high molecular wt, Na-alginate, Iota-carrageenan commercial grade type II, DPPH and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt (Ferrozine) were purchased from Sigma-Aldrich, USA. EDTA, ferric chloride, ferrous chloride, *Egypt. J. Rad. Sci. Applic.*, Vol. 24, No. 1 (2011)

Potassium ferricyanide and Trichloroacetic acid were supplied from BDH, India. Other reagents and solvents were of analytical grade. The number average molecular wt of the degraded polymers were determined by GPC, 1100 Agilent instrument, USA and using a calibration curve using polyethylene oxide standards. UV-Vis spectrophotometer was carried by using Jasco V-560, Japan. FT-IR spectrophotometer was carried out in the form of KBr pellets by using JASCO FT-IR 6300, Japan.

Evaluation of antioxidant activity of unirradiated and irradiated natural polymers was investigated using different methods such as measurement of scavenging activity on DPPH radicals (Yamaguchi *et al.*, 1998), the reducing power (Yen and Duh 1993) and the chelating effect on ferrous ion (Dinis *et al.*, 1994). Chitosan (in 1% acetic acid) or Na-alginate or carrageenan solutions with different concentrations (mg/ml) were irradiated by ^{60}Co γ -rays at different doses of 10, 20 and 30 kGy at dose rate of 3.52 kGy/ h at the time of experiments. The irradiation facility was constructed by the NCRRT, Nasr City, Cairo, Egypt.

Results and Discussion

The changes in the number average molecular wt of chitosan, Na-alginate and carrageenan in aqueous solution using GPC after treating with gamma irradiation at different doses were shown in Fig. 1. There is a rapid decrease in the number average molecular wt of chitosan, Na-alginate and i-carrageenan. As the irradiation dose increases, the number average molecular wt of polysaccharides decrease. The number average molecular wt of chitosan, Na-alginate and carrageenan solution subjected to 10kGy, decreased from 1.9×10^6 , 2.4×10^6 and 4.3×10^6 (Da) to 3.8×10^5 , 1.1×10^5 and 2.5×10^5 (Da), respectively. With increasing the irradiation dose to 30kGy, the number average molecular wt reduced to 9.9×10^3 , 9.2×10^3 and 1.2×10^4 (Da), respectively. Polysaccharides are typical degradable materials under ionizing radiation which undergo degradation reactions through the β -(1-4) glycosidic bond cleavage resulting in the reduction of its average molecular wt (Leonhardt *et al.*, 1985). UV-Vis. spectra of unirradiated and irradiated chitosan, Na-alginate and carrageenan were illustrated in Fig. 2. For the irradiated chitosan, two absorption peaks at 275 and 315 nm were observed. The intensity of these peaks increased with increasing the irradiation dose. These two peaks may be due to

the presence of unsaturated carbonyl and carboxyl groups. The obtained results are consistent with those reported by Ulanski and Rosiak (1992). For the irradiated Na-alginate and carrageenan, a new absorption band at 275 nm and the peak intensity increase with increasing the irradiation dose. It can be assigned to carbonyl group or hydroxyl group formed after the main chain scission of the polysaccharide (Nagasawa *et al.*, 2000).

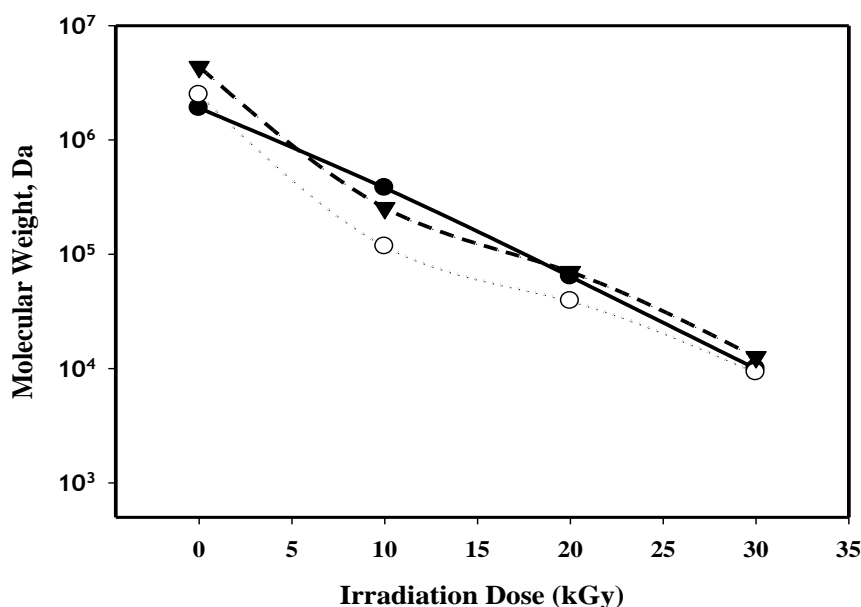


Fig. 1. The average molecular weights of (●) chitosan, (○) Na-alginate and (▼) carrageenan in aqueous solution after γ -irradiation at different doses.

FT-IR spectra of unirradiated and irradiated chitosan, Na-alginate and carrageenan were shown in Fig. 3. FT-IR spectrum of chitosan (Fig. 3. A, curve a) shows distinctive absorption bands appeared at 3440, 1638, 1598, 1387, 1156 and 1097 cm^{-1} corresponds to $-\text{OH}$ and $-\text{NH}_2$ groups, stretching $\text{C}=\text{O}$ of amide group, N-H bend, NH of amide, asymmetric bridge-O-stretch and skeletal vibration involving the C-O stretch, respectively are characteristic of saccharine structure. For the irradiated chitosan (Fig. 3. A curves b, c & d), it was observed that as the irradiation dose increases, the intensity of the absorption peak at 1602 cm^{-1} assigned to the N-H bend vibration of $-\text{NH}_2$ increases and shift to low wavenumber. This may be due to the decreasing of inter- or intra- molecular hydrogen bonding between the $-\text{OH}$ and $-\text{NH}_2$ groups.

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Additionally, another characteristic peak at 1638 cm^{-1} , which could be disturbed by carbonyl groups of chitosan, was gradually shifted to 1645 cm^{-1} , while its intensity increased with an increase in the γ -ray irradiation dose. Also, the intensity of the peak at 1385 cm^{-1} assigned to C–O stretch vibration increased and moved toward the higher wavenumber as the irradiation dose increases. The vibrational band at 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 in the molecular chains of chitosan.

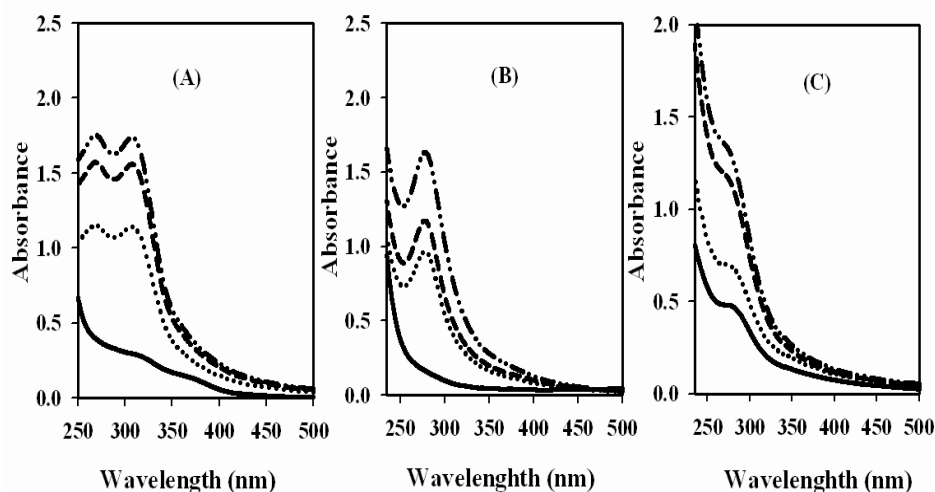


Fig. 2. UV-Vis spectra of (A) chitosan, (B) Na-alginate and (C) carrageenan, (—) unirradiated polysaccharide and irradiated polysaccharide at 10 kGy (---), 20 kGy (---) and 30 kGy (-·-·-).

In the spectrum of Na-alginate (3B, curve a), the peaks at 3390 , 1620 and 1095 - 1035 cm^{-1} are attributed to hydroxyl, C–O–O and C–O–C groups, respectively (Sartori *et al.*, 1997). Peak at 1610 cm^{-1} for $-\text{COONa}$ of Na-alginate was taken as the reference peaks due to the fact that carboxyl groups do not change after degradation. The spectrum of degraded Na-alginate (Fig. 3. B, curves b, c & d) exhibited most of the characteristic absorption peaks of native alginate. The scission of glycosidic bonds leads to the formation of hydroxyl group, which is manifested as an increase in the ratio of hydroxyl group peak at 3425 cm^{-1} . For unirradiated carrageenan spectrum (Fig. 3. C, curve a), the broad band between 855 - 805 cm^{-1} indicated to the sulphate groups that attached at the C-4 position of galactose.

The band at 934 cm^{-1} was attributed to the presence of 3,6-anhydrogalactose. The vibrational band at 1075 cm^{-1} corresponds to the ether bond in the pyranose ring. The peak at 1262 cm^{-1} was attributed to the asymmetric stretching of S=O. The absorption bands at 2941 and 3460 cm^{-1} due to C-H stretch groups and hydroxyl (OH) groups, respectively.

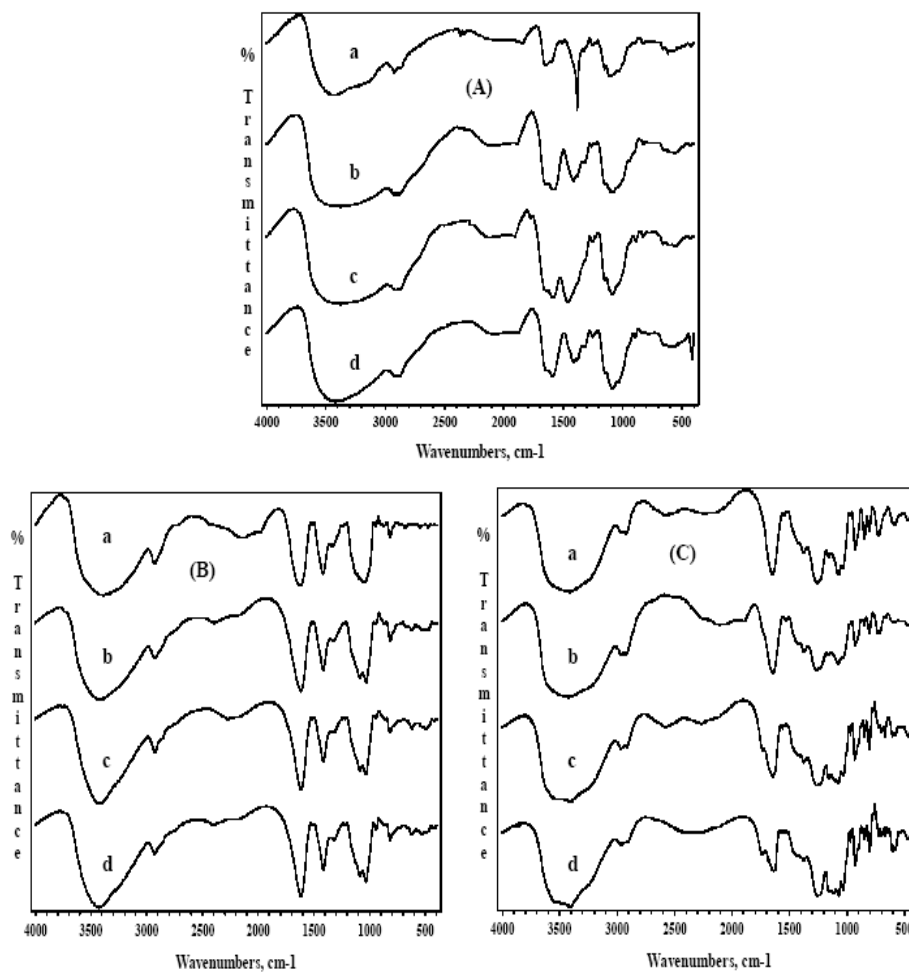


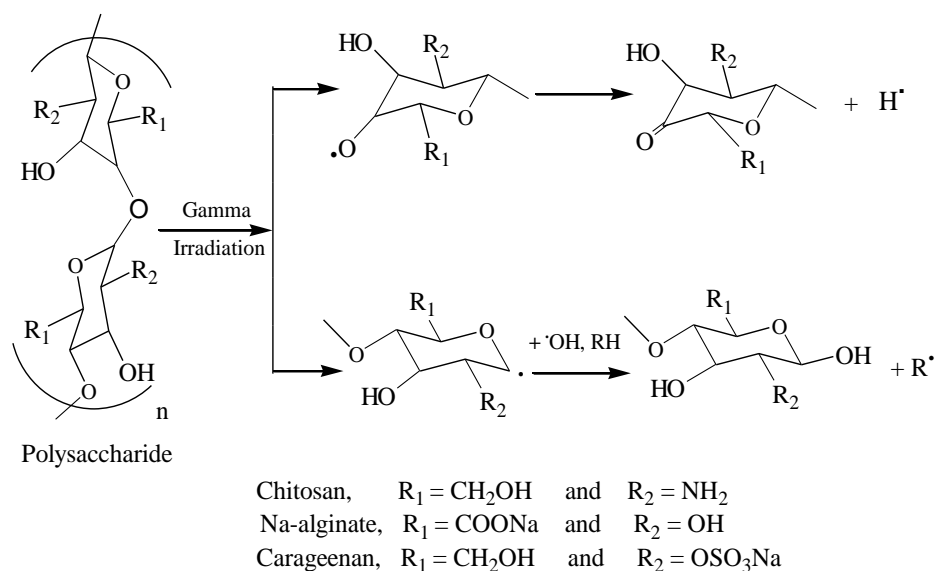
Fig. 3. FT-IR spectra of (A) chitosan, (B) Na-alginate and (C) carrageenan, (a) unirradiated polysaccharide and irradiated polysaccharide at (b) 10kGy, (c) 20kGy and (d) 30kGy.

The spectrum of irradiated carrageenan (Fig. 3. curves b, c & d) exhibits most of the characteristic absorption peaks of unirradiated carrageenan but there

is a new band appeared at 1732 cm^{-1} due to glycoside bonds cleavage and formation of -C=O groups.

From the data obtained by UV-Vis. and FT-IR studies, the proposed mechanism of degradation of chitosan, Na-alginate and carrageenan irradiated in liquid state could be illustrated in Schema. 1.

DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. Further, it is well accepted that the DPPH free radical scavenging by antioxidants is due to their hydrogen-donating ability (Chen and Ho, 1995). DPPH scavenging effect (%) of chitosan, Na-alginate and carrageenan with different concentrations and irradiation doses were measured and the results were depicted in Fig. 4. In general DPPH radical scavenging effect (%) of chitosan, Na-alginate and carrageenan increased with increasing the polysaccharide concentration to a certain extent about 1 mg/ml and then it levelled off even with further increase in the concentration. Also, an increase in the DPPH radical scavenging effect (%) was observed by increasing the irradiation dose of polysaccharides.



Schema. 1. Proposed mechanism of radiation induced degradation of chitosan, Na-alginate and carrageenan.

The results revealed that the DPPH radical scavenging effect (%) of investigated polysaccharides followed the order chitosan > Na-alginate > carrageenan. The DPPH scavenging effect (%) for the unirradiated chitosan, Na-alginate and carrageenan at a concentration of 0.5 mg/ml was 5, 3.2 and 3.1 (%), respectively. Meanwhile, using 30kGy irradiation dose, the DPPH scavenging effect (%) became 60.2, 52.7 and 51.1 (%), respectively. The DPPH scavenging effect (%) of chitosan irradiated at 30kGy is nearly the same as that of ascorbic acid which is 62.5 (%). IC₅₀ of DPPH radical scavenging effect (%) was determined to be 0.154, 0.359, and 0.438 mg/ml for chitosan, Na-alginate and carrageenan irradiated at 30kGy, respectively as compared by that of ascorbic acid 0.140 mg/ml. These results revealed that the irradiation of chitosan enhances its antioxidant activity.

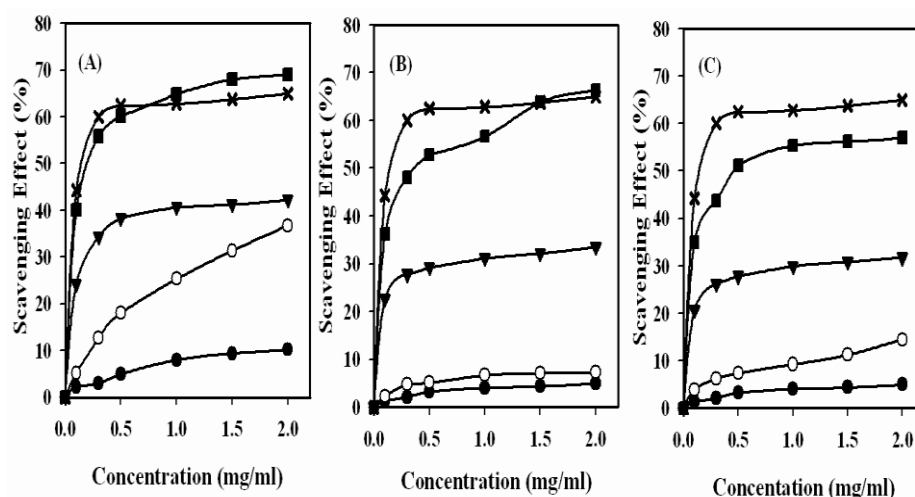


Fig. 4. Scavenging effect (%) of (A) chitosan, (B) Na-alginate and (C) carrageenan; irradiated at different doses on DPPH radicals. (x) ascorbic acid as reference, (●) unirradiated polysaccharides and the irradiated polysaccharides at (◻) 10kGy, (▼) 20kGy and (■) 30kGy.

The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. The reducing power of unirradiated and irradiated chitosan, Na-alginate and carrageenan at different doses as a function of concentration comparing with ascorbic acid (positive control) was shown in Fig. 5. Low molecular wt polysaccharides showed high reducing power, and the

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reducing power increased with increasing the polysaccharides concentration. The reducing power of irradiated chitosan is much higher than that of Na-alginate and carrageenan. At 2 mg/ml concentration, the reducing power of unirradiated chitosan, Na-alginate and carrageenan was 0.3319, 0.3504 and 0.3146, respectively. Using 30kGy irradiation dose and 2mg/ml polysaccharide concentration, the reducing power of chitosan, Na-alginate and carrageenan became 1.3574, 1.2380 and 0.9377, respectively. Meanwhile, the reducing power of ascorbic acid was 1.48. The reducing power properties are generally associated with the presence of reducing agent. The role of reducing agents is to exert antioxidant action by breaking the free radicals chain by donating a hydrogen atom (Gordon 1990).

The chelating effect (%) of unirradiated and irradiated chitosan, Na-alginate and carrageenan at different doses as a function of concentration using EDTA as positive control was shown in Fig. 6. Low molecular wt polysaccharides showed high chelating ability. The chelating ability increased with the increase of polysaccharides concentration.

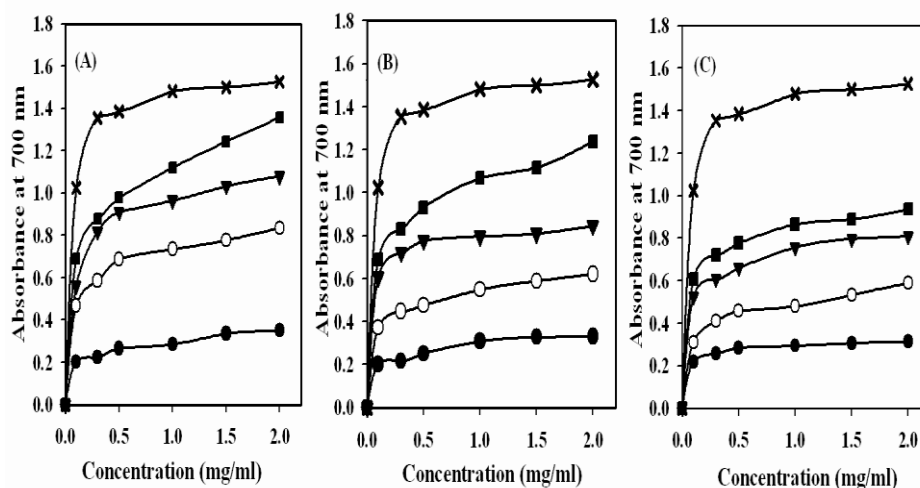


Fig. 5. Reducing power of (A) chitosan, (B) Na-alginate and (C) carrageenan; irradiated at different doses. (x) ascorbic acid as reference, (●) unirradiated polysaccharides and the irradiated polysaccharides at (○) 10kGy, (▼) 20kGy and (■) 30kGy.

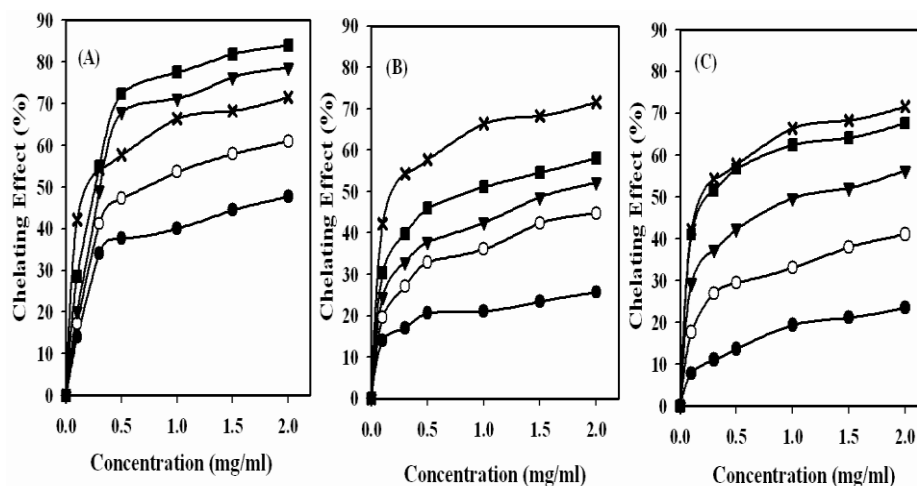


Fig. 6. Chelating effect (%) of (A) chitosan, (B) Na-alginate and (C) carrageenan; irradiated at different doses. (x) EDTA as reference, (●) unirradiated polysaccharides and the irradiated polysaccharides at (○) 10kGy, (▼) 20kGy and (■) 30kGy.

The results revealed that the irradiated chitosan had the highest chelating ability. The affinity of chitosan to chelate Fe^{2+} ion mainly comes from the presence of amino groups, which contain lone electron pairs that help to form chitosan- Fe^{2+} complexes (Guzman *et al.*, 2003). At 1 mg/ml concentration, the chelating (%) of unirradiated; chitosan, Na-alginate and carrageenan was 40, 21.1 and 19.3 (%), respectively, in comparison with that of EDTA which gave 66.4 (%). Using 30kGy irradiation dose, the chelating (%) of chitosan, Na-alginate and carrageenan became 77.5, 51.1 and 62.4 (%), respectively. IC_{50} of the chelating effect (%) was 0.2293, 0.8023 and 0.238 for chitosan irradiated at 20kGy, Na-alginate irradiated at 30kGy and carrageenan irradiated at 30kGy, respectively. The chelating effect (%) of 30kGy irradiated chitosan was higher than that of Na-alginate and/ or carrageenan.

Conclusions

A rapid decrease in the number average molecular wt of chitosan, Na-alginate and carrageenan was obtained during the radiation induced degradation process. FT-IR, UV-Vis. spectroscopy revealed that the main polysaccharide chain structure was almost remained after degradation. The increase in the irradiation dose of γ -ray treatment and concentration of polysaccharides

increased the antioxidant activities. The results showed that the chitosan oligosaccharide possessed the highest scavenging effect on DPPH radicals rather than Na-alginate and carrageenan oligosaccharides. Also, the 30kGy irradiated chitosan had nearly the same antioxidant activity of ascorbic acid at the same concentration and can be used as a source of natural antioxidant for food shelf life extension.

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دراسة مقارنة على خصائص مضادات الأكسدة لبعض البوليمرات الطبيعية المكسرة بالإشعاع الجامي

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قسم البحوث الإشعاعية لكيمياء البوليمرات ، المركز القومي لبحوث و تكنولوجيا الإشعاع ، ص.ب. 29 مدينة نصر، و*قسم الكيمياء ، كلية العلوم ، جامعة عين شمس ، القاهرة ، مصر.

تم دراسة تأثير الإشعاع على بعض البوليمرات الطبيعية مثل الكيتوزان و ألجينات الصوديوم و الكاراجينان وذلك للحصول على بوليمرات ذات أوزان جزيئية صغيرة باستخدام الإشعاع الجامي المؤين. وقد تم توصيف الشكل التركيبي لهذه البوليمرات ذات الأوزان الجزيئية الصغيرة باستخدام مطياف الأشعة فوق بنفسجية (UV-Vis) ، و مطياف الأشعة تحت الحمراء (FT-IR) و تعيين الوزن الجزيئي باستخدام الجبل الكروماتوجرافي المنفذ (GPC). أوضحت النتائج أن الوزن الجزيئي ينقص بزيادة الجرعة الإشعاعية وأن الشكل التركيبي لهذه البوليمرات الطبيعية لم يتغير بعد عملية التكسير بالإشعاع. أجريت دراسة مقارنة على خصائص مضادات الأكسدة للجزيئات الصغيرة من الكيتوزان و ألجينات الصوديوم و الكاراجينان ذات الأوزان الجزيئية المختلفة من حيث تأثير الإلتهام على DPPH ذات الشقوق الحرة و قياس قوة الإختزال و نشاطية الترابط مع أيون الحديد و المقارنة بمادة مؤكسدة (حامض الأسكوربيك). و كذلك أظهرت النتائج أن عملية التكسير بالإشعاع لمثل هذه البوليمرات تحسن النشاطية المضادة للأكسدة خاصة البوليمرات ذات الأوزان الجزيئية الصغيرة. كما أظهرت النتائج أن الخواص المضادة للأكسدة للكيتوزان المشع أفضل و أكبر من ألجينات الصوديوم و الكاراجينان. فعند استخدام الجرعة الإشعاعية 30 كيلو جراي فإن التركيز المثبط (IC_{50}) لتأثير الإلتهام على DPPH ذات الشقوق الحرة لكل من الكيتوزان و ألجينات الصوديوم و الكاراجينان و حامض الأسكوربيك هي 0.154 ، و 0.359 ، و 0.438 ، و 0.140 على التوالي.