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Radiation-induced degradation of chitosan for possible use as a growth promoter in agricultural purposes

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ABSTRACT

Controlling the degradation of chitosan by gamma ray from a ^{60}Co source in the in presence of initiators such as ammonium per-sulfate and hydrogen peroxide, was investigated. The factors affecting the degradation process such as irradiation dose, type of initiator and its concentrations were studied. The efficiency of these methods was verified by viscometric and GPC analysis and the average molecular weight of degraded chitosan were determined. The irradiation degradation in the presence of chemical initiator was much more appropriate from economical point of view because it reduced the irradiation doses required for degradation. Characterization of degraded polymer by FT-IR spectroscopy, UV–vis spectroscopy, XRD, ESR and TGA analysis was investigated. The water-soluble chitosan separated from degraded chitosan prepared at different irradiation doses showed a strong effect on the growth of Faba bean plant and can be used in agriculture fields as a growth promoter.

1. Introduction

Chitosan usually has high molecular weight polysaccharide and strong network of intermolecular or intramolecular hydrogen bonds (Huang, Zhai, Peng, Li, & Wei, 2007). Chitosan one of the most important marine polysaccharide has no toxic effect on human beings and has many peculiar biological activities such as immunity, norcholesterol and antibacterial (Chandy & Sharma, 1992; Felse & Panda, 1999). Chitosan is derived from the most abundant natural polymer chitin; is composed of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units. Chitosan usually has high molecular weight polysaccharide and strong network of intermolecular or intramolecular hydrogen bonds (Huang et al., 2007). Also chitin/chitosan are extensively studied and used widely in food processing to increase viscosity as emulsifiers in medicine and in cosmetics as carriers for drugs, enzymes, tissue, wound dressing, hair spray, skin care cream, etc. Chitosan is a biodegradable polysaccharide having amine groups explains its unique properties among biopolymers. The structure of chitosan results in poor solubility in most organic solvent and makes it chemically inert for derivatization. Since only a few chemical modifications can be made in chitosan-carboxylic acid aqueous solution (acetic acid or citric acid) most reactions of chitosan are carried out in heterogeneous systems of organic solvents (Chirachanchai, Lertworasiriku, & Tachaboonyakiat, 2001). So it is of increasing interest to degrade chitosan to low molecular weight under appropriate conditions and then compare the relationship between biological activity and molecular weight in order to improve its solubility. Degradation of chitosan is usually used, turning chitosan into one with low molecular weight which exhibits good water solubility. The water-soluble chitosan with low molecular weight has some special biological, chemical and physical properties which are different from that of the ordinary chitosan such as antibacterial activity (Zheng & Zhu, 2003), antifungal activity (Jeon, Park, & Kim, 2001) and antitumor activity (Qin, Du, Xiao, Li, & Gao, 2002). Chitosan has very positive impact on plants growth; all plants with chitosan had better developed roots and shoots in the field of agriculture (Chmielewski et al., 2007). Due to many unique properties such as biocompatibility, biodegradability, nontoxicity and nonantigenicity; chitosan has been widely applied in medicine, biotechnology, water treatment, agriculture and food science (Majeti & Kumar, 2000). In agriculture, chitosan has been used in seed, leaf, fruit and vegetable coating (Devlieghere, Vermeulen, & Debevere, 2004), to protect plants against microorganisms (Pospieszny, Chirkov, & Atabekov, 1991). A variety of techniques including acid hydrolysis (Jeon & Kim, 2000), enzymatic methods (Ilyina, Tikhonov, Albulov, & Varlamov, 2000), ultraviolet degradation (Wang, Huang, & Wang, 2005) and gamma radiation processing can be used to prepare chitosan oligomers (Hai, Diep, Nagasawa, Yoshii, & Kume, 2003; Kang, Dai, Zhang, & Chen, 2007; Wasikiewicz, Yoshii, Nagasawa, Wach, & Mitomo, 2005). In the last decades oxidative degradation with hydrogen peroxide has been studied (Shao, Yang, & Zhong, 2003).

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In this paper, the degradation of chitosan using ionizing radiation in the presence of ammonium per-sulfate and hydrogen peroxide was investigated. The degraded chitosan was characterized by UV–vis spectroscopy, XRD, ESR and TGA analysis. The effect of the radiation degraded chitosan oligomers on the growth of some plants is examined.

2. Materials and methods

2.1. Materials

Chitosan, degree of deacetylation not less than 85%, of high molecular weight was supplied from Aldrich, Hydrogen peroxide (H_2O_2) 30% Loba chemie, ammonium per-sulfate BDH limited Poote 98% England, were used as received, Faba bean seeds from Elwady company for production of seeds and agricultural crops, Egypt were used.

2.2. Preparation of degraded chitosan

Degraded chitosan was prepared by mixing chitosan (powder) with 10 (wt.%) initiator with a few drops of distilled water to obtain chitosan in the paste form. The chitosan in the paste form was packaged in polyethylene bags then subjected to gamma irradiation ^{60}Co source with various absorbed doses from 20 to 200 kGy.

2.3. Separation (fractionation) of water-soluble chitosan

The irradiated chitosan in presence of 10 (wt.%) potassium per-sulfate at different absorbed doses from 40 to 200 kGy is heated in distilled water at 70 °C in a water bath for 2 h and then filtrated to descant the water insoluble parts. The filtrate was precipitated by adding few drops of methanol, and then filtrated, and the obtained filtrate was dried in an oven at 50 °C for 1 h to get powder water-soluble chitosan.

2.4. Determination the molecular weight of degraded chitosan

The weight-average molecular weights of the degradable polymers were determined by two methods:

2.4.1. The vicometric method

The weight-average molecular weights of chitosan can be determined by measuring the intrinsic viscosity of polymer solution $[\eta]$ of chitosan samples using an ubbelohde viscometer (Ilyina, Tatarinova, & Varlamov, 1999; Lim, Khor, & Koo, 1998; Ulanski & Rosiak, 1992). The solvent used is 0.3 M acetic acid – 0.2 M sodium acetate solution taking $K = 0.076$ ml/g and $\alpha = 0.76$ (Rinaudo, Milas, & Dung, 1993) using ubbelohde glass capillary viscometer and on the basis of the Mark-Houwink equation $[\eta] = kM^{\alpha}$ where $[\eta]$ is the intrinsic viscosity of the polymers, k and α are the polymer/solvent interaction constants and M is the average molecular weight.

2.4.2. Gel permeation chromatography (GPC) method

The number-average molecular weight (Mn) and weight-average molecular weights (Mw) were measured by gel permeation chromatography (GPC) 1100 Agilant instrument equipped with organic and aqueous GPC-SEC start up kits with a flow rate of 2 ml/min, maximum pressure 150 bar, minimum pressure 5 bar, injection volume 50 μL and column temperature thermostat 25 °C. The eluent was monitored by a refractive index detector of optical unit temperature 25 °C and peak width 0.1 min. polymer concentration was 0.1 (w/%). The molecular weights were determined from a calibration curve using polyethylene oxide standards for aqueous systems. The method used for determining the number-average molecular weight (Mn) and weight-average molecular weights (Mw) followed (Li, Du, & Liang, 2007; Liu, Bao, Du, Zhou, & Kennedy, 2006).

2.5. FT-IR spectroscopy

FT-IR study was carried out using Matton 1000, Unicam, Cambridge spectrophotometer, England in the range of 400–4000 cm^{-1}.

2.6. UV–vis spectroscopy

UV–vis study was carried out using Jasco V-560, Japan, in the range from 190 to 900 nm.

2.7. X-ray diffraction (XRD) studies

X-ray diffraction patterns were obtained with a XRD-DI series, Shimadzu apparatus using nickel-filter and Cu–K a target.

2.8. Electron spin resonance (ESR)

ESR signals were recorded at room temperature by using a Bruker EMX spectrometer (X-band) product of Bruker, Germany. The polymer powder is placed in the sample tubes. The stretching direction of the films was either parallel or perpendicular to the axis of the magnetic field.

2.9. Thermal gravimetric analysis (TGA)

Perkin Elmar TGA system under Nitrogen atmosphere 10 ml/min was used. The temperature range was from ambient temperature to 600 °C at flow rate ≈10 ml/min.

2.10. Plantation

To investigate the effect of degraded chitosan on Faba bean plants, the concentration of 100 ppm was used. The field can be divided into eight separate lines. After plantation of ages 30, 60 and 90 days, each line of plants are sprayed by 100 ppm solution of (b) untreated chitosan, (c) irradiated chitosan at 200 kGy, (d) chitosan treated by ammonium per-sulfate at 40 kGy, (e) chitosan treated by ammonium per-sulfate at 80 kGy, (f) chitosan treated by ammonium per-sulfate at 120 kGy, (g) chitosan treated by ammonium per-sulfate at 160 kGy and (h) chitosan treated by ammonium per-sulfate at 200 kGy. The growth rate of the plants by these treatments is compared with control (a) (untreated plant).

3. Results and discussion

3.1. Effect of gamma irradiation on chitosan

Fig. 1 shows the changes in the viscosity average molecular weights of chitosan in powder form after treating with gamma irradiation at different doses. As the irradiation dose increases, the viscosity average molecular weight of chitosan molecules decreases. The decrease in the molecular weight is very fast up to 120 kGy. Thereafter, the degradation rate was very low. It was reported that the polysaccharides are typical degradable materials under ionizing radiation based on the reduction of molecular weight (Leonhardt et al., 1985; Nakamura, Ogiwara, & Phillips, 1985). The chitosan molecules undergo degradation reactions through the β-(1–4) glycosidic bond cleavage resulting in the reduction of its viscosity average molecular weights.

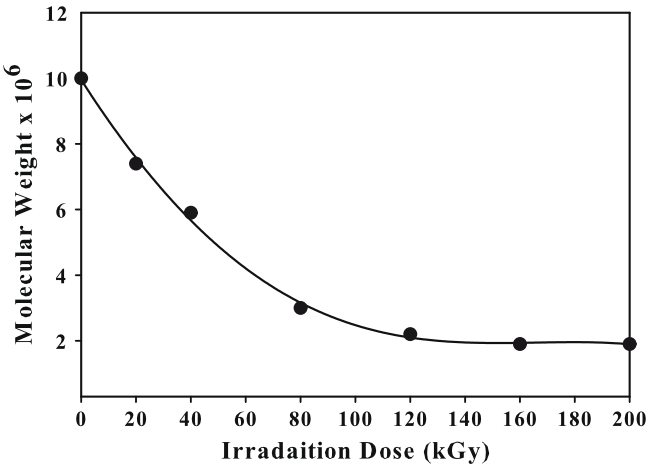

Fig. 1. Changes in the viscosity average molecular weight of chitosan powder after exposing to gamma irradiation at the dose rate 6.7 kGy/h.

3.2. Effect of gamma irradiation on the degradation process of chitosan in the presence of some initiators

Radiation can induce degradation of natural polymers like chitosan and the dose required for degrading of solid polysaccharides ranged from 200–500 kGy. In fact, from the economic point of view these doses are not accepted; the cost is high. Therefore, trails have been made to reduce the cost of degradation process of solid polysaccharides by using some initiators to reduce the dose required for degradation. Fig. 2 shows the effect of temperature (70 °C) and γ-irradiation (80 kGy) in the presence of different initiators on degradation of chitosan as a function of the change in the viscosity average molecular weight. It was observed that the addition of such initiators to chitosan during the thermal or radiation treatment accelerates the degradation process. The rate of the degradation of chitosan molecular weight treated with ionizing radiation in the presence of chemical initiator is much higher than that treated with ionizing radiation alone. The presence of hydrogen peroxide or potassium per-sulfate or ammonium per-sulfate enhances the radiation degradation process of chitosan. Also, from the Fig. 2, the ammonium per-sulfate is the most effective imitator on degradation of chitosan to obtain lower molecular weight ranged from 3×10^6 to 1.3×10^5 at the dose of 80 kGy.

3.3. Effect of initiator concentration

Fig. 3 shows the effect of ammonium per-sulfate concentration on degradation process of chitosan treated with gamma irradiation at 80 kGy as a function of the change in its viscosity average molecular weight. The degradation of chitosan increases with increasing the ammonium per-sulfate concentration. To obtain low viscosity average molecular weight very effectively from 3×10^6 to

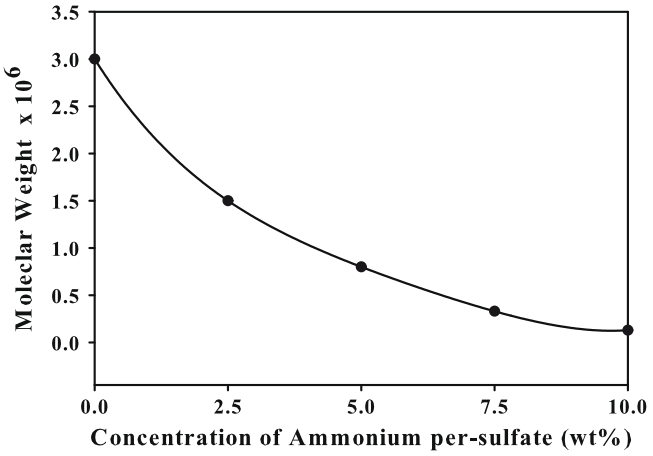

Fig. 3. Effect of ammonium per-sulfate concentration on degradation process of chitosan, irradiation dose 80 kGy, dose rate 6.7 kGy/h.

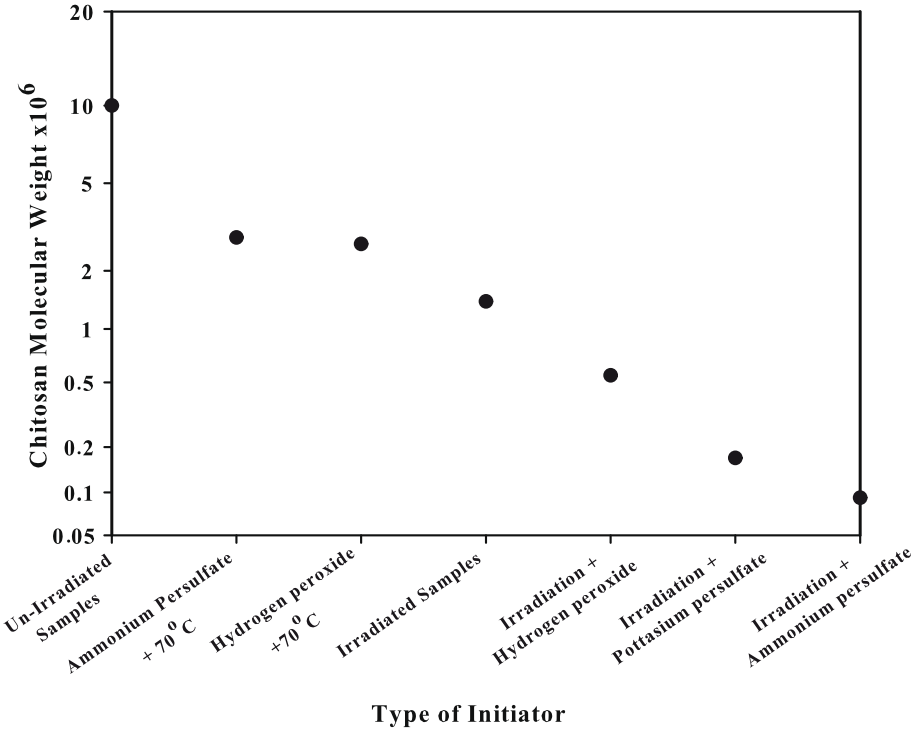

Fig. 2. Effect of some salt addition on degradation process of chitosan treated by gamma irradiation at 80 kGy at the dose rate 6.7 kGy/h or thermal heating at 70 °C.

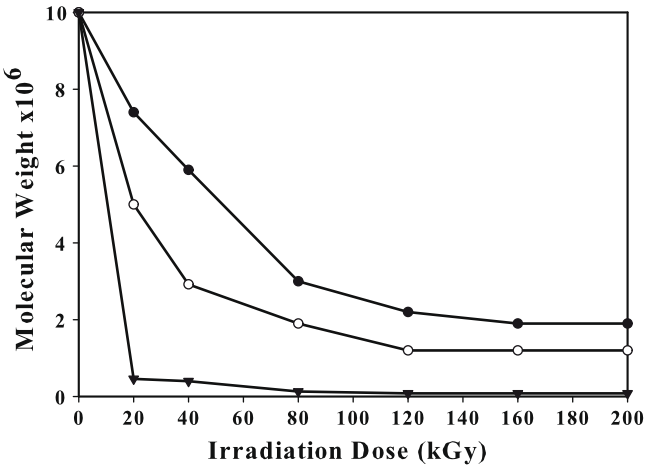

Fig. 4. The changes in the viscosity average molecular weight of pure chitosan exposure to : (●) γ-irradiation (○) γ-irradiation + 10% H_2O_2 (v/w) and (▼) γ-irradiation + 10% APS (w/w) at the dose rate 6.7 kGy/h.

1.3×10^5 at 10 (wt.%) ammonium per-sulfate concentration. The chitosan main chain is composed of pyranose rings linked by β-(1,4) glycosidic bond. The presence of ammonium per-sulfate initiate free radicals attack the C-1 or C-4 carbon and then break the adjacent glycosidic bond (C–O–C) in the main chain.

3.4. Effect of irradiation dose on degradation of chitosan

3.4.1. Viscometric method

Fig. 4 shows the changes in the viscosity average molecular weights measured by viscometric method as a function of the irradiation dose of pure chitosan powder and that mixed with (w/w) 10% hydrogen peroxide or 10 (wt.%) ammonium per-sulfate. It is observed that the addition of initiators to the polysaccharides during the irradiation process accelerates the degradation process. Meanwhile, in all treatment conditions with increasing the irradiation dose, the decrease in the viscosity average molecular weight of chitosan as well as the increase in the rate of degradation process were observed. The highest degradation rate is obtained when the chitosan irradiated in the presence of ammonium per-sulfate (APS). A wide variety of lower molecular weights and shorter chain polymers or oligomers could be produced depending on the treatment conditions. Using 80 KGy irradiation dose reduces the average molecular weight of chitosan molecules from 1×10^7 to 3×10^6 but the irradiation of chitosan molecules at 40 kGy in the presence of APS is enough to reduce the average molecular weight from 1×10^7 to 1.3×10^5. From the results obtained it can be concluded that the chitosan was depolymerized, their molecular weights decrease and the degradation of the backbone occurs in a random fashion.

3.4.2. Gel permeation chromatography (GPC) method

Fig. 5 shows the GPC elution curves of pure, irradiated chitosan at 120 kGy and that irradiated in the presence of 10% hydrogen peroxide or 10 (wt.%) ammonium per-sulfate as a function of retention time (min). The large molecular weight molecules of large size appear with low retention time and the lower molecular weight molecules of small size appears at high retention time.

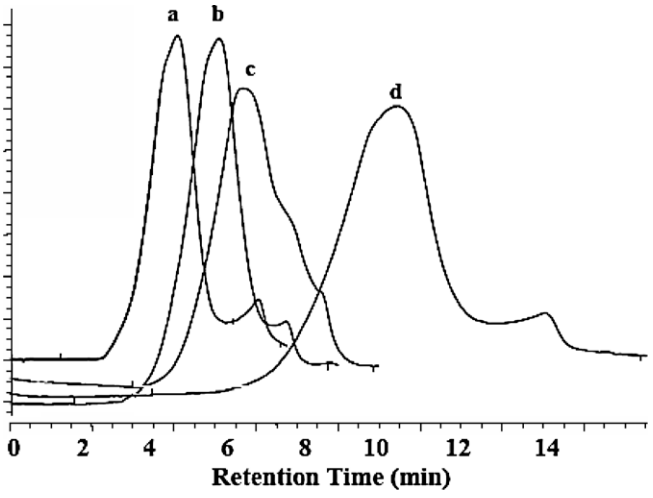

Fig. 5. GPC elution curves of chitosan as a function of retention time (a) blank, (b) irradiated at 120 kGy, (c) irradiated at 120 kGy in the presence of 10% APS and (d) irradiated at 120 kGy in the presence of H_2O_2.

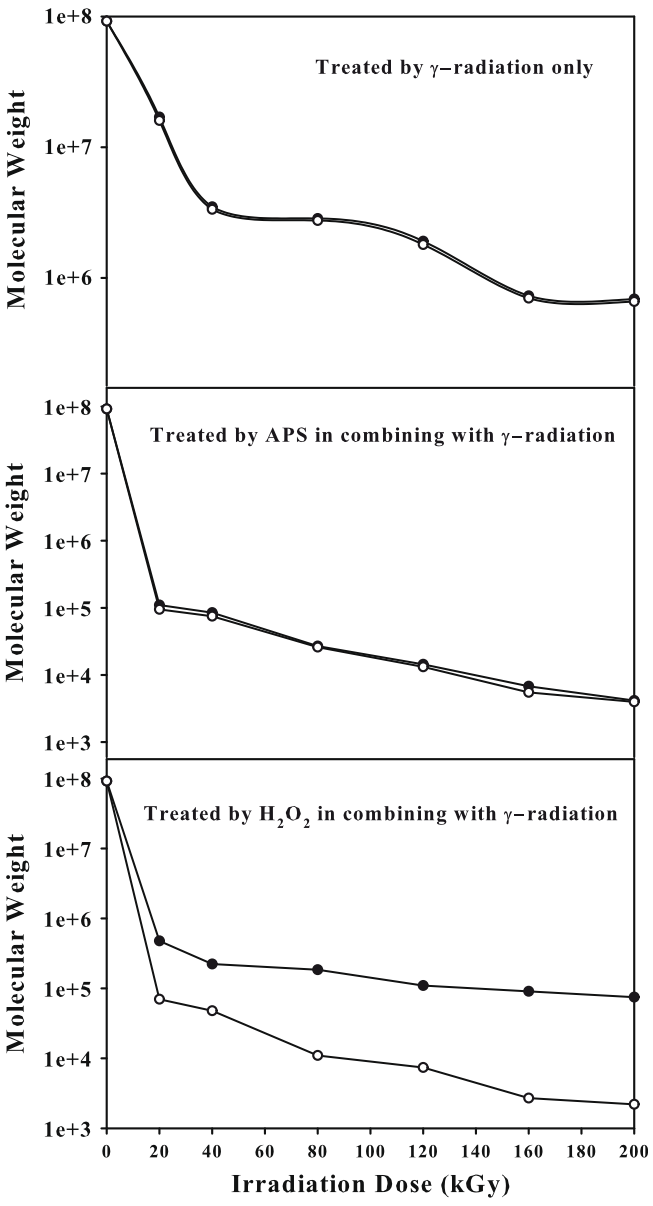

Fig. 6. The changes in the number-average molecular weight of chitosan measured by GPC when exposed to gamma irradiation in the presence of 10% H_2O_2 (v/w) or 10% APS (w/w) (●) after irradiation and (○) after irradiation and storage for 2 months.

Fig. 6 shows the changes in the number-average molecular weights of chitosan using GPC as a function of irradiation dose for chitosan when exposed to gamma irradiation in the presence and absence of initiators (10% H_2O_2 or 10% APS) and storage for 2 months. A steep reduction in the number-average molecular weights of chitosan was observed up to 20 kGy in presence of ammonium per-sulfate and hydrogen peroxide and gradually increased as the irradiation dose increased. The storage effect on the molecular weight of irradiated chitosan in the presence of 10% H_2O_2 or 10% APS was studied. The degradation reactions of these polymers depend on the amount of free radicals formed during the irradiation process. The free radicals decay occurred in chitosan irradiated in the presence of APS is higher than that occurred in chitosan irradiated in the presence of H_2O_2. The free radicals formed in the presence of H_2O_2 are very low decay and therefore, the degradation process is in continuous with passing of time. However, in case of chitosan irradiated in the presence of APS the termination reaction may be fast as a result, stable fragments or oligomers with shorter chains and molecular weights can be obtained.

3.5. Characterization of degraded chitosan

3.5.1. FT-IR spectroscopy

Fig. 7a and b shows the IR spectra of untreated and 120 kGy irradiated chitosan. The spectrum of degraded chitosan exhibited most of the characteristic adsorption peaks of unirradaited chitosan but with some differences: the vibrational band at 1100 cm^{-1} that corresponds to the ether bond in the pyranose ring was weakened, which indicates that the rupture of the β-glycosidic bonds may have led to several effects on the amount and distribution of glycosidic bonds in the molecular chains of chitosan by a strong, broad band centered at 3440 cm^{-1}. The bands at 1602 and 599 cm^{-1} correspond to the binding vibrations of the amido groups. The sharp bands at 1602 and 599 cm^{-1} become stronger, and the peak at 599 cm^{-1} moves toward the higher wavenumbers (Wang, Huang, & Wang, 2005). In addition, the N–H stretching vibration moved toward lower wavenumbers which indicates that the intermolecular and intramolecular hydrogen bonds of chitosan were weakened and its crystallinity was reduced after degradation. A new peak at 1634 cm^{-1} which is assigned to absorbance of C=O, indicating that the carboxyl or carbonyl groups do exist (Shao et al., 2003). These changes in spectra also confirmed that carbonyl or carboxyl groups were formed and partial amino groups were eliminated during the radiation degradation process of chitosan. Furthermore, the profiles of spectra a and b are similar so that we can conclude that the main polysaccharide chain structure was remained during degradation process.

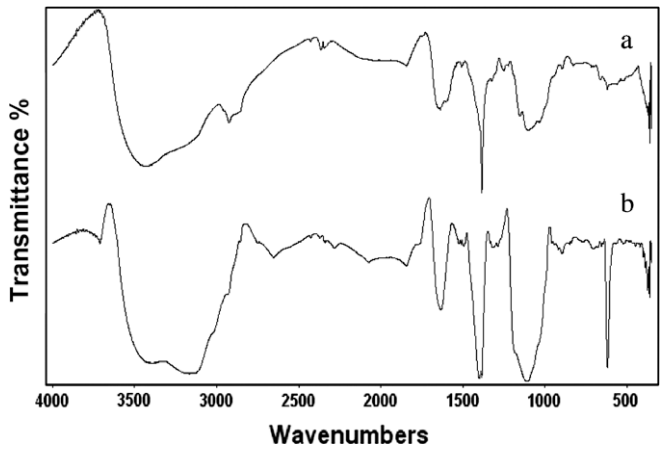

Fig. 7. FT-IR spectra of (a) unirradaited chitosan and (b) water-soluble chitosan obtained from the treatment of chitosan by 10(wt.%) ammonium per-sulfate and 120 kGy irradiation dose.

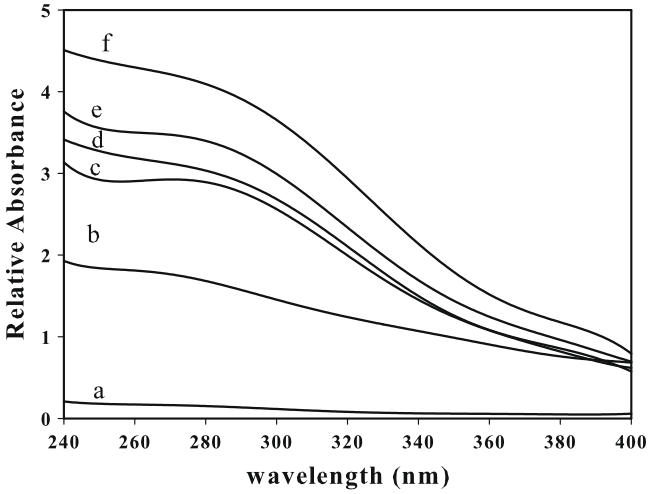

Fig. 8. UV–vis spectra of (a) pure chitosan and that treated with 10(wt.%) ammonium per-sulfate at different irradiation doses of (b) 40, (c) 80, (d) 120, (e) 160 and (f) 200 kGy at the dose rate 6.7 kGy/h.

3.5.2. UV-vis spectroscopy

Fig. 8 shows the UV–vis spectra of pure chitosan and that treated with 10 wt.% ammonium per-sulfate at different irradiation doses of 40, 80, 120, 160 and 200 kGy. For treated chitosan a strong absorption band was evident around 280–300 nm which may be caused by the $n \rightarrow \delta^*$ transition for the amino groups of chitosan and may also assigned to the $n \rightarrow \pi^*$ transition for the carbonyl or carboxylic groups. The peak intensity increased with increasing the irradiation dose. This peak can be ascribed to carbon–oxygen double bonds (carbonyl groups) formed after the main chain scission of chitosan and hydrogen abstraction reaction followed by the ring opening (Ulanski & Rosiak, 1992).

3.5.3. X-ray diffraction (XRD)

Fig. 9 shows the X-ray diffraction patterns of untreated chitosan and that irradiated in the presence of 10 wt.% APS at dose of 40, 120 and 200 kGy. Untreated and treated chitosan exhibited two characteristic peaks at $2\theta = 10.4°$ and $2\theta = 19.8°$ which coincided

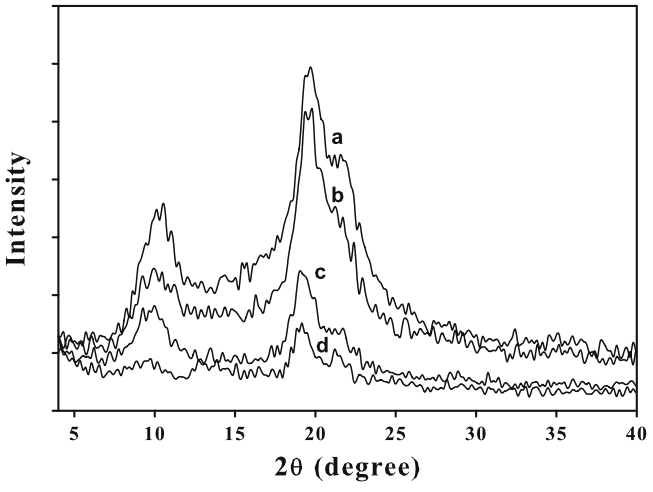

Fig. 9. XRD spectra of (a) pure chitosan and that treated with 10(wt.%) ammonium per-sulfate irradiated at different doses of (b) 40 kGy; (c) 120 kGy and (d) 200 kGy at the dose rate is 6.7 kGy/h.

with the pattern of the L-2 polymorph of shrimp chitosan as reported (Hasegawa, Isogai, Onabe, Usuda, & Atalla, 1992). There is change in the intensity of the peaks with increasing the irradiation dose. Radiation degradation did not destroy crystal structure of chitosan completely up to 200 kGy. For chitosan after radiation degradation the first peak at $2\theta = 19.8°$ is much less intensive and the peaks at $2\theta = 10.4°$ may be disappeared which is due to the destruction of hydrogen bonding between amino groups and hydroxyl groups in chitosan (Kim & Lee, 1993). The results show that -radiation degradation of chitosan caused destruction of the crystal structure. The water-soluble fraction became amorphous. Thus, it was assumed that the degradation first took place preferentially in the amorphous region and then proceeded very moderately from the edge to the inside of the crystalline. With deeper degradation, the crystalline structure was destroyed and the crystallinity decreased.

3.5.4. Thermal gravimetric analysis (TGA)

Fig. 10 and Table 1 show the TGA curves and the weight loss percent of unirradiated and irradiated chitosan in the presence of 10 wt.% APS at different irradiation doses. As seen from the results in 10, for chitosan, the weight loss took place in two stages. The first one starts below 100 °C is assigned to loss of water. Water molecules interact with two different polar groups and that the interactions with amine groups are weaker than those with hydroxyl groups. Since a considerable amount of water is released at temperatures below 150 °C, The second stage starts at 240 °C and reaches a maximum at 350 °C corresponds to the decomposition (thermal and oxidative) of chitosan, vaporization and elimination of volatile products (Nieto, Peniche-Covas, & Padron, 1991; Tirkistani, 1998). It was found that there is no significant change in the thermal stability of chitosan irradiated at different irradiation doses in comparison each other and small difference for that of pure chitosan comprising with irradiated ones. Pyrolysis of polysaccharides starts by a random split of the glycosidic bonds, followed by a further decomposition forming acetic and butyric acids and a series of lower fatty acids, where C_2, C_3 and C_6 predominate (Nieto et al., 1991).

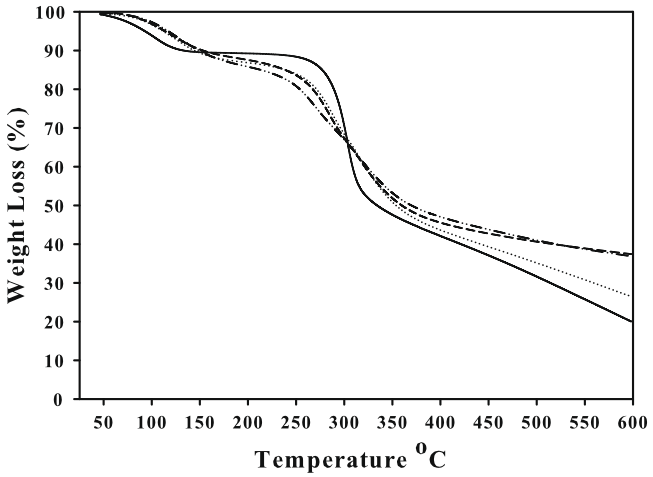

Fig. 10. TGA diagram of (—) pure chitosan and that treated with 10 (wt.%) ammonium per-sulfate at different irradiation doses of (·····) 40 kGy; (—) 120 kGy and (·–·–·–·) 200 kGy at the dose rate 6.7 kGy/h.

3.5.5. Electron spin resonance analysis (ESR)

Fig. 11 shows the ESR spectra of irradiated chitosan in the presence and absence of 10 wt.% ammonium per-sulfate (APS) or 10%

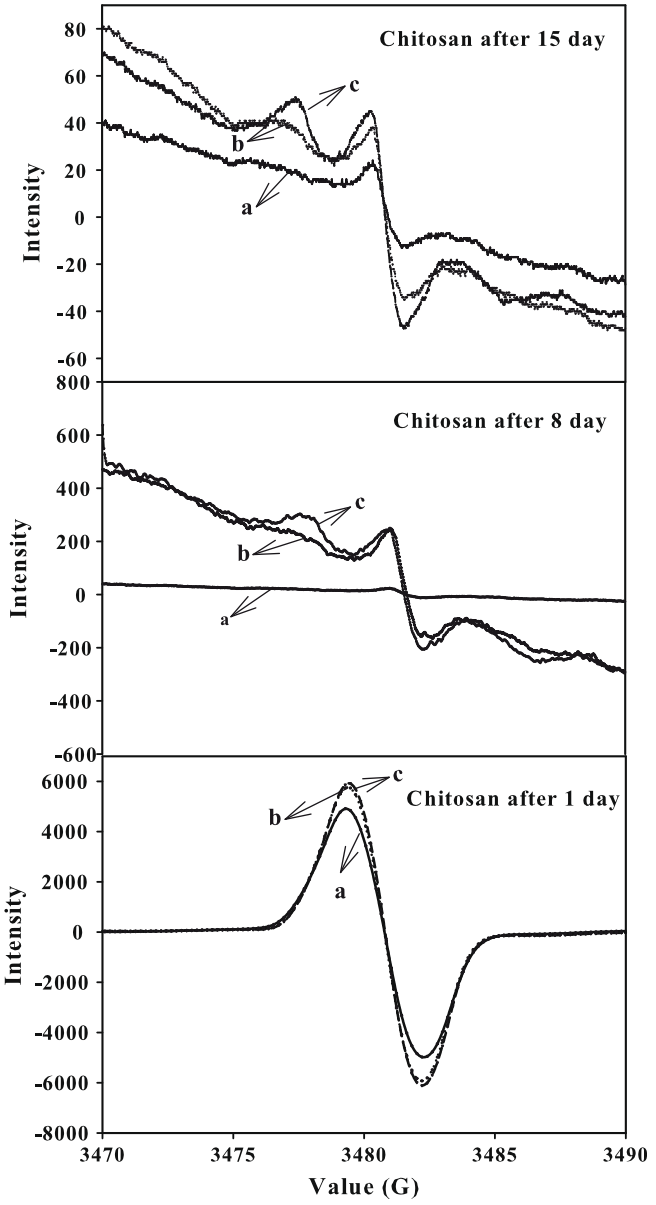

Fig. 11. ESR spectrum of (a) chitosan irradiated at dose of 120 kGy, (b) irradiated in the presence of 10 (wt.%) ammonium per-sulfate, (c) irradiated in the presence of 10% hydrogen peroxide (v/w) were measured after 1, 8 and 15 days at the dose rate 6.7 kGy/h.

Table 1
The thermal stability and weight loss percent for untreated and irradiated chitosan at different irradiation doses.

Sample	Weight loss (%)				
	25–100 °C	100–200 °C	200–250 °C	250–300 °C	300–350 °C
Pure chitosan	6.2	10.8	11.7	29.5	52.5
Chitosan + APS at 40 kGy	6.5	12.51	13.4	30.3	50.2
Chitosan + APS at 120 kGy	5.9	13.6	21.5	41	51.8
Chitosan + APS at 200 kGy	6.1	16	26.1	40.8	50.5

Table 2
Effect of spraying 100 ppm of chitosan and that mixed by 10% wt APS at different irradiation doses on crop yield of faba bean.

Sample	Factor						
	Plant height (cm)	Number of pods/ plant	Number of pods/ Plant/m^2	Number of plant/m^2	Biological yield fad (ardab)	Straw yield fad (ardab)	Seed yield/ fad (ardab)
Control	99.51	17.68	162	32.94	22.98	14.87	7.71
Blank	90.13	17.92	164	32.2	22.83	14.77	8.06
200 kGy	90.24	17.7	162	32.27	22.82	14.71	8.11
APS 40 kGy	111.53	21.87	210	44.84	24.13	15.19	8.94
APS 80 kGy	121.61	23.85	233	50.79	28.58	18.71	9.87
APS 120 kGy	125.18	24.55	241	52.9	32.34	21.62	10.72
APS 160 kGy	117.91	23.12	225	48.6	41.31	31.69	9.62
APS 200 kGy	109.98	21.572	207	43.92	39.15	30.07	9.08

H_2O_2 (v/w) after storing for 1, 8 and 15 days. From the results of ESR, after storing for one day, it is obvious that, the intensity of free radicals formed for irradiated chitosan and that irradiated in the presence of with 10 (wt.%) APS or 10% H_2O_2 (v/w) are the same. After storing for 8 and 15 days of irradiation, the peak of free radicals for these natural polymers irradiated in the presence of hydrogen peroxide still present with lower intensity if compared with that formed after one day. The free radical intensity of chitosan irradiated in the presence of H_2O_2 is higher than that for irradiated natural polymer in absence or presence of ammonium per-sulfate at the same dose. The intensity of free radicals for irradiated natural polymer in absence or presence of ammonium per-sulfate decreased and disappeared after storage for 8 days. This indicates that the radicals formed in the presence of hydrogen peroxide is successive and continuously. Also, ESR studies of the irradiated samples revealed that the decay rate of the radicals in the presence of hydrogen peroxide is much lower than that for samples irradiated in the presence of ammonium per-sulfate.

4. Effect of radiation degraded chitosan on growth of faba bean plants

Fig. 12 shows the effect of chitosan of different molecular weights on growth of faba bean plant. The test field results in Fig. 12 showed that the treatment of the faba bean plant with irradiated chitosan from 80 to 160 enhances not only the plant growth but also the productivity. The increase in plant performance by using degraded chitosan suggested its possible use in agriculture purposes as growth promoters. The same results as reported (Chmielewski et al., 2007). In addition the chitosan acts as antifungal compound (Pospieszny et al., 1991) and confirmed that a minor amount of chitosan has profound effects on the growth and development of orchid plant tissue. Chitosan has a unique combination of attractive characteristics: it stimulates plant growth, provides plant protection and environmental friendly being of biological origin and is easy biodegradable by soil microorganisms. Table 2 describe plant height (cm), number of pods/ plant, number of pods/plant/m^2, number of plant/m^2, biological yield fad. (ardab), straw yield fad. (ardab) and seed yield/fad. (ardab) for the plant spraying by 100 ppm of water-soluble chitosan. The treatment of faba bean plant by degraded chitosan has positive effect on seed yields (ardab/fad) which gives faba bean seed yield of 10.72 (ardab/fad) if compared with control plant which gives 8.7 (ardab/fad).

5. Conclusion

The degradation process of chitosan can be carried out by gamma irradiation in presence of ammonium per-sulfate or hydrogen peroxide. The ammonium per-sulfate is the most effective imitator on degradation of chitosan to obtain the lower molecular weight from 3×10^6 to 1.3×10^5 at the dose of 80 kGy. The water-soluble fraction can be separated after the degradation process and its quantity increased by the irradiation dose. The results of XRD show that chemical-radiation degradation of chitosan caused destruction of its crystal structure. ESR analysis indicated that the reaction by hydrogen peroxide is in successive and continuously. The degraded chitosan has a positive effect not only on plant growth but also on the productivity of Faba plant, which suggested its possible use in agriculture purposes as growth promoters.

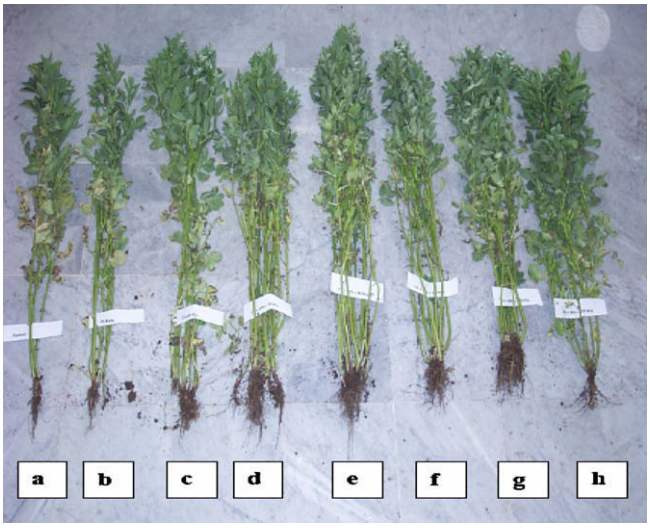

Fig. 12. Effect of chitosan on growth of faba bean plant (a) control, (b)treated with unirradaited chitosan, (c) rradiated chitosan at 200 kGy, (d) irradiated at 40 kGy + APS, (e) irradiated at 80 kGy + APS, (f) irradiated at 120 kGy + APS, (g) irradiated at 160 kGy + APS and (h) irradiated at 200 kGy + APS.

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