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Study on antibacterial starch/chitosan blend film formed under the action of irradiation

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Abstract

Starch/chitosan blend films were prepared by irradiation of compression-molded starch-based mixture in physical gel state with electron beam (EB) at room temperature. The influence of chitosan and radiation on the properties of the prepared films was investigated. The tensile strength and the flexibility of starch film were improved largely after incorporation of 20% chitosan into starch film. X-ray diffraction and scanning electron microscope analyses of starch/chitosan blend films indicated that there was interaction and microphase separation between starch and chitosan molecules. Furthermore, in order to produce a kind of antibacterial films, the starch/chitosan blend film was irradiated, and the antibacterial activity of the starch/chitosan blend films against *Escherichia coli* (*E. coli*) was measured via optical density method. After irradiation, there is no obvious change in the structure of starch/chitosan blend films, but antibacterial activity was induced even when the content of chitosan was only 5% due to the degradation of chitosan in blend films under the action of irradiation.

Keywords: Starch; Chitosan; Irradiation; Blend film; Antibacterial

1. Introduction

Starch is renewable and biodegradable polysaccharides (Lenz, 1993). Usually native starch contains about 30% amylose, 70% amylopectin and less than 1% lipids and proteins from plant. Biodegradable starch-based plastics, such as starch/cellulose, starch/PVA (poly-vinyl alcohol), etc. have recently been investigated due to great potential markets in agricultural foils, garbage or composting bags, food packaging, fast food industry as well as biomedical fields (Funke, Bergthaller, & Lindhauer, 1998; Ishigaki, Kawagoshi, Ike, & Fujita, 1999; Lorcks, 1998; Lourdin, Valle, & Colonna, 1995; Marques, Reis, & Hunt, 2002; Psomiadou, Arvanitoyannis, & Yamamoto, 1996; Reis & Cunha, 1995; van Soest, Benes, & De Wit, 1996). Radiation technique is a kind of important method of material modification. In our laboratory radiation modification of starch-based plastics has been investigated (Zhai, Yoshii, & Kume, 2003). The ductility and tensile strength of the starch-based plastics were improved due to the crosslinking reactions of amylopection molecules of starch in physical gel state induced by radiation.

Chitosan consists of 2-deoxy-2-amino anhydroglucose residues, is obtained by N-deacetylation of chitin, which is the second most naturally occurring biopolymer after cellulose. Since chitosan is a biodegradable polysaccharide having amine groups, many papers have recently been published concerning utilization of chitosan as functionalized polysaccharides (Francis Suh & Matthew, 2002; Ishihara et al., 2002; Ravi Kumar, 2000; Ruel-Gariépy, Chenite, Chaput, Guirguis, & Leroux, 2000; Zhai, Lin, Li, He, & Wei, 2001). Study on functionalized blend films such as cellulose- chitosan, PVA-chitosan blend film, etc. has been reported (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto, & Aiba, 1997; Hasegawa, Isogai, Kuga, & Onabe, 1994). Recently, the antibacterial and antifungal activities of chitosan have been followed with great interest. The molecular weight and amino group of chitosan have the obvious influence on its antibacterial activity

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(Le, Nagasawa, Matsuhashi, Ishioka, Ito, & Kume, 2001; Liu, Guan, Yang, Li, & Yao, 2001).

In previous works, radiation-induced antibacterial activity of chitosan has been investigated in our laboratory (Le et al., 2001). Radiation dose has obvious influence on antibacterial activity of chitosan due to the degradation of chitosan. Here chitosan will be incorporated into starch system to prepare antibacterial starch/chitosan blend films under the action of irradiation.

2. Experimental

2.1. Materials

Starch (cornstarch) was supplied by Gunei Chemical Industrial Co. Ltd, Japan. Chitosan (8B, Mv: 1.5×10^5) was purchased from Katokichi Chemical Co., Japan. Glycerol, acetic acid and dimethyl sulfoxide (DMSO) were obtained from Kanto Chemical Co. Inc. Japan.

2.2. Formation of antibacterial starch/chitosan blend films

The preparation of pure starch film was described in detail in the previous paper (Zhai et al., 2003) Here chitosan solution was prepared by dissolving chitosan in aqueous solution of acetic acid (chitosan:acetic acid = 5:4). Then starch powder mixed with glycerol was further mixed homogeneously with the above chitosan solution to form 15% starch and chitosan semisolid gel-like mixtures by heating at 100 °C for 2 h. The content of glycerol is 20%, which was expressed as mass percent ratio of glycerol to chitosan and starch. The gel-like mixtures in hot state were cold pressed to make wet starch/chitosan films (thickness of wet film: 0.5 mm).

In order to produce a kind of antibacterial films, the wet films formed through above methods were irradiated further at room temperature by EB with beam current of 1 mA and acceleration energy of 2 MeV generated by the Cockroft Walton Electron Beam Accelerator. Afterwards, wet starch/chitosan films were dried naturally at room temperature to gain starch/chitosan films.

2.3. Tensile strength and elongation at break

Starch or starch/chitosan blend films dried at room temperature were cut into dumbbell (ASTM D-1822-L), and then tensile strength and elongation at break were measured at strograph-R1 Material Tester (Toyoseiki Co. Ltd, Japan) with a crosshead speed of 50 mm/min.

2.4. Water absorption

Starch or starch/chitosan blend films dried naturally in room temperature were immersed directly in distilled water at room temperature to the equilibrium (24 h). The content of water adsorbed in starch/chitosan films was calculated as

$$\mathrm{CWA} = \frac{W_e - W_d}{W_d}$$

Where W_e is the weight of starch/chitosan films at the adsorbing equilibrium and W_d is the dry weight of starch/chitosan films.

2.5. X-ray diffraction measurements

X-ray diffraction experiments were performed for the films dried naturally in room temperature by Rigaku X-ray diffractomer. The X-ray source was Ni-filtered Cu Kα radiation (40 kV, 30 mA). Film samples were scanned from 5 to 40° 2θ at a scanning rate of 4° 2θ/min.

2.6. SEM analyses

The morphological structures of the films dried naturally in room temperature were studied by JSM-5600 scanning electron microscope of JEOL, Japan. The dried samples were coated with gold, and then observed and photographed.

2.7. Antibacterial assessment of starch/chitosan films

Antibacterial activity of the starch/chitosan blend films against *E. coli* B/r was evaluated by using the optical density method. In order to eliminate the influence of acids on antibacterial activity of starch/chitosan blend films, acetic acids remaining in the composite films were neutralized by washing with a 0.2 mol l^{-1} Na_2CO_3 aqueous solution followed by distilled water. 0.05 g dry films was added into 5 ml medium (Nutrient broth from Difico, Michigan, USA) including two percent *E. coli* B/r culture where *E. coli* B/r grew in a logarithmic growth phase and then incubated at 37 °C for 48 h. During the incubation, the turbidity of the medium was measured at 650 nm every 2 h. All of the operations were done in aseptic condition or by aseptic technique.

3. Results and discussion

3.1. Mechanical properties of starch/chitosan blend films

In our previous experiment, the formation of starch-based films in physical gel state has been investigated, and manifested that radiation had an important influence on the formation of starch-based plastic. The films without irradiation shrunk and broke into the fragment after drying naturally at room temperature. But when starch-based film in physical gel state was irradiated, an intact, smooth starch-based sheet was formed after drying at the same condition. In the meantime the ductility and tensile strength of the starch-based plastics were improved due to the crosslinking

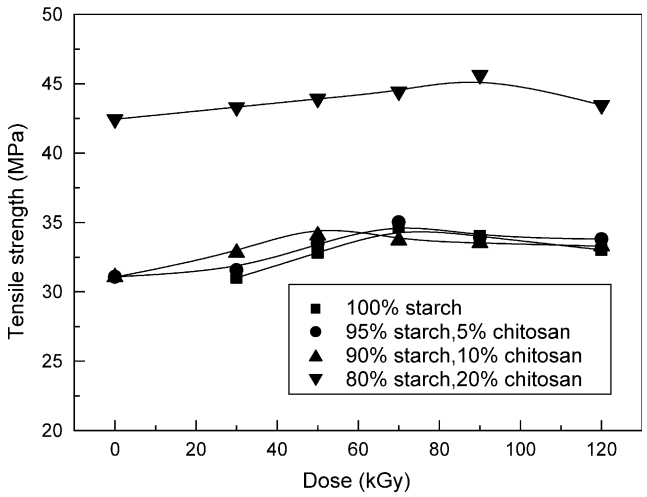

Fig. 1. The tensile strength of starch/chitosan blend films after drying naturally at room temperature.

reactions of amylopection molecule of starch in physical gel state induced by radiation (Zhai et al., 2003). Here starch/chitosan blend films were investigated, after chitosan was incorporated into starch film, the formation condition and ductility of starch-based films were improved obviously. Without irradiation, an intact, smooth starch/chitosan blend film was also formed after drying naturally at room temperature. The change of tensile strength with the content of chitosan and radiation dose was shown in Fig. 1. The tensile strength of the films increased largely after incorporating 20% chitosan into starch film. Because it is very difficult to form homogeneous starch/chitosan mixture with higher content of chitosan. Twenty percent chitosan is maximum value used in starch/chitosan mixture in this experiment. In cellulose–chitosan blend films, the tensile strength of the films was maximum at a chitosan content of 10–20% due to the presence of interaction between cellulose and chitosan molecules in the films (Hasegawa et al., 1994; Hasegawa, Isogai, Onabe, Usada, & Atalla, 1992).

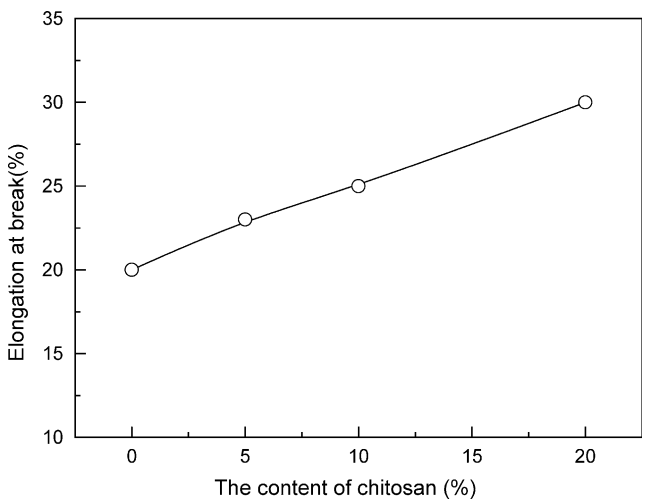

Fig. 2. Elongation at break of starch/chitosan blend films after drying naturally at room temperature (50 kGy).

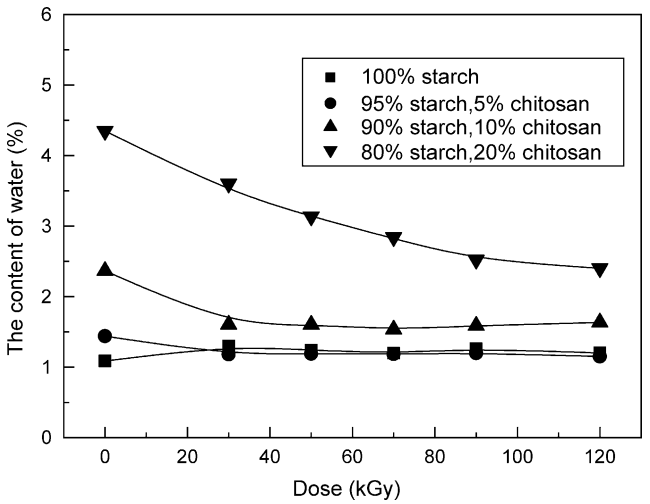

Fig. 3. The content of water absorbed in starch/chitosan blend films.

The structure of starch is the same as that of cellulose except for the configuration. So it can be thought that there is interaction between starch and chitosan molecules in starch/chitosan blend films, especially between amylose of starch and chitosan molecules because amylose is easier to mix with chitosan than amylopectin.

The change of elongation at break with the content of chitosan was shown in Fig. 2. Elongation at break increased with the content of chitosan. It was not found that there was an obvious change in elongation at break with the dose. The change of elongation at break of starch/chitosan blend films with the content of chitosan is also similar to that of cellulose–chitosan blend films.

3.2. Water absorption and wet strength of starch/chitosan films

Water sensitivity is another important criterion for many practical applications of starch-based plastics. In order to

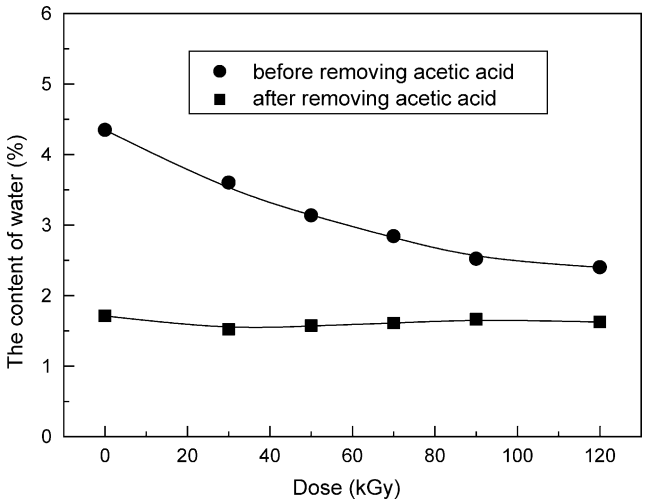

Fig. 4. The content of water absorbed in starch/chitosan blend films after neutralizing acetic acid.

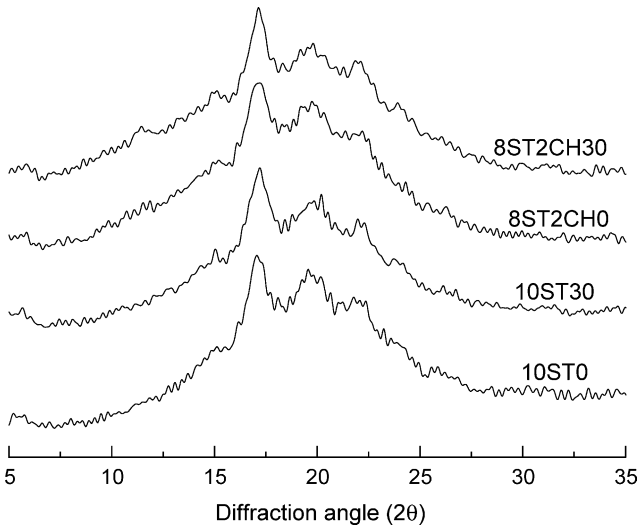

Fig. 5. X-ray diffraction patterns of starch and starch/chitosan blend films.

analyze the hydrophilic character of starch/chitosan blend films prepared by irradiation, the properties of water absorption were investigated here. The results were shown in Fig. 3. The content of water adsorbed in starch/chitosan blend films increased with adding chitosan, but decreased lightly with dose. The former attribute to the presence of acetic acid, because chitosan can dissolve in acetic acid. In the presence of acid, chitosan in the films is easy to swell, so the content of water adsorbed in starch/chitosan blend films increased. After neutralizing acetic acid by soaked in 0.2 mol l^{-1} Na_2CO_3, water absorption in starch/chitosan films decreased obviously. (Fig. 4). The decrease of water absorption after irradiation mainly attribute to the dissolution of hydrophilic chitosan with low molecule produced by the degradation of chitosan under irradiation (Zhai et al., 2001). After adsorbing water, the tensile strength of starch/chitosan films decreased largely, but wet strength of starch/chitosan film is obviously higher than that of pure starch film.

3.3. X-ray analyses of starch/chitosan blend films

X-ray diffraction patterns of starch films (control) and starch/chitosan blend films were shown in Fig. 5. X-ray diffraction of starch had been reported (Cheetham & Tao, 1998; Gernat, Rodosta, Anger, & Damaschun, 1993), and there are three different conformations in crystalline parts of starch. Here starch film (100% starch, 0 kGy, 10ST0) showed a typical B-type pattern, with strong diffraction peak at around 17° 2θ and a few small peaks at around 2θ of 20°, 22°. After irradiation at 30 kGy, the crystalline pattern and the degree of crystallinity of starch films (100% starch, 30 kGy, 10ST30) did not nearly change. According to Hasegawa's results, diffraction peak of chitosan is at around 10 and 20° 2θ (Hasegawa et al., 1992). In this work, there was no obvious difference in X-ray diffraction patterns between starch films (10ST0) and starch/chitosan blend films (80% starch, 20% chitosan, 0 kGy, 8ST2CH0) except for that crystallity degree of starch films decreased lightly

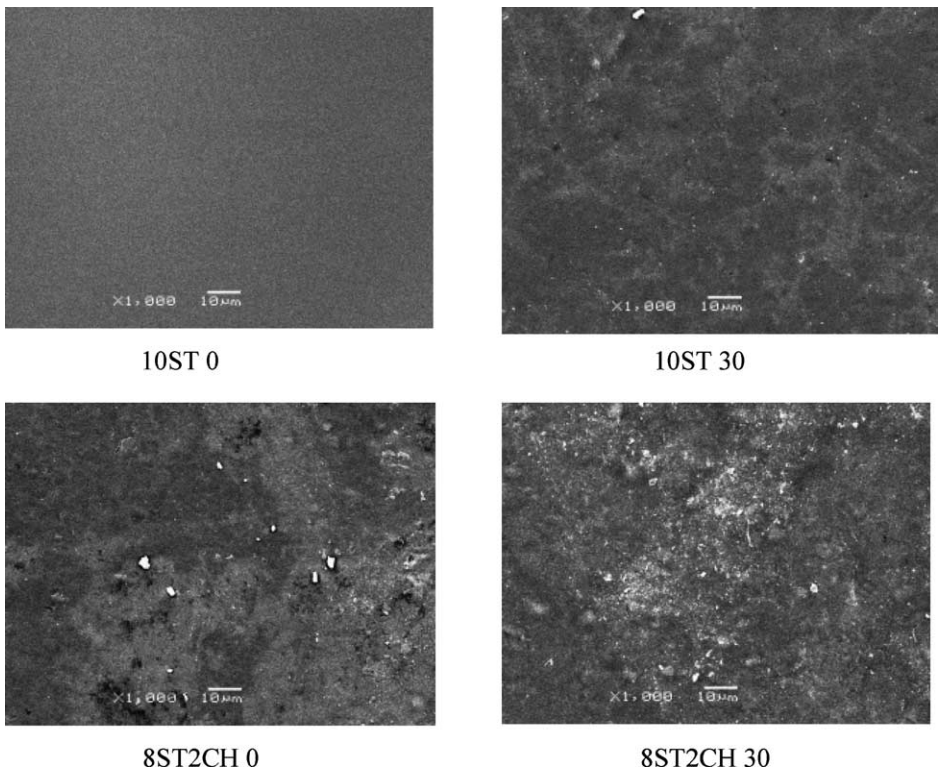

Fig. 6. SEM micrographs of the surface of starch and starch/chitosan blend films.

from 9.9 to 7.1% after blending with 20% chitosan. The experiment results suggested that there was interaction between starch and chitosan molecules in the films. Because if starch and chitosan have low compatibility in the blend films, each polysaccharide has its own crystal region in the blend film, and X-ray diffraction patterns are expressed as simply mixed patterns. In other polysaccharide blend films, the same phenomenon on X-ray diffraction pattern also had been observed (Hasegawa et al., 1992; Yu, Du, & Zheng, 1999). The crystalline pattern and the degree of crystallinity of starch/chitosan blend films (80% starch, 20% chitosan, 30 kGy, 8ST2CH30) also did not nearly change after irradiation at 30 kGy.

3.4. SEM observation of starch/chitosan blend films

Scanning electron microscope (SEM) microphotographs of the surfaces of starch films (control) and blend films were shown in Fig. 6. 10ST0 in Fig. 6 displayed the surface of unirradiated starch film, which is relatively flat. After irradiation at 30 kGy, there is microphase separation (10ST30) on the surface of starch film due to the presence of crosslinking networks of amylopectin molecules of starch (Zhai et al., 2003). 8ST2CH0 in Fig. 6 showed that there are heterogeneous surfaces for unirradiated starch/chitosan blend films due to the microphase separation of starch and chitosan component. Chitosan microdomains dispersed within starch matrix, which is similar to the surface of cellulose/CM–chitosan blend films (Li, Zhuang, Liu, Guan, & Yao, 2002). When starch/chitosan blend films were irradiation at 30 kGy, the sizes of chitosan microdomains in the blend film (8ST2CH30) decreased due to the degradation of chitosan under irradiation.

3.5. Antibacterial activity of starch/chitosan blend films

Since biomedical materials have to sterilize or prevent from many of microorganisms, the antibacterial activity will

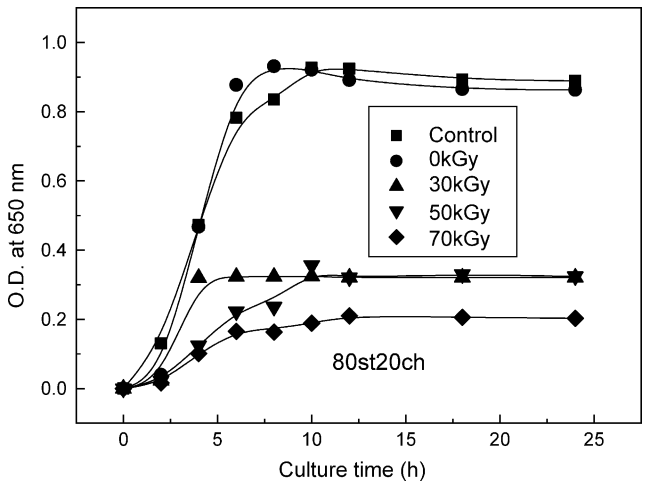

Fig. 7. The change of antibacterial activities of starch/chitosan blend films with radiation dose (80% starch, 20% chitosan).

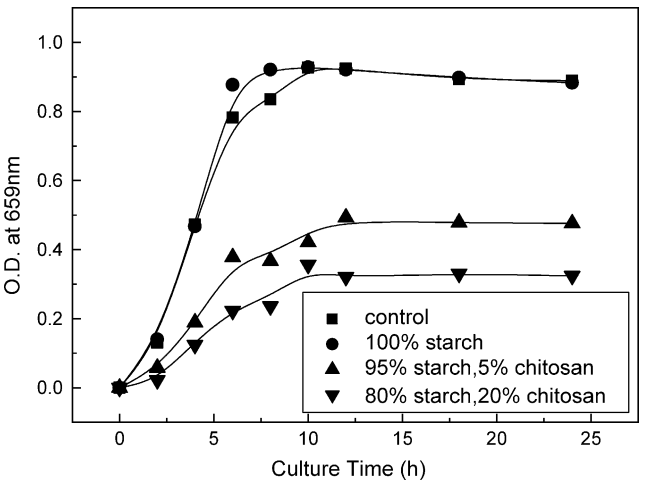

Fig. 8. The change of antibacterial activities of starch/chitosan blend films with the content of chitosan (50 kGy).

be the most valuable properties in the field of biomedical application. Antibacterial activity of chitosan could be induced by irradiation (Le et al., 2001; Zhai et al., 2001). Here, the starch/chitosan blend films made by irradiation were tested to confirm whether chitosan could induce the satisfying antibacterial activity to the blend films. Fig. 7 and 8 demonstrated the curves of optical density (OD) versus culture time for the blend films against *E. coli*. After 30 kGy it can be seen that the blend films exhibited significant antibacterial activities compared to the pure medium or unirradiated blend films (Fig. 7). OD of the blend films decreased with irradiation does, which indicated that antibacterial activity of blend films improved with the increasing of dose. Fig. 8 showed that even the content of chitosan in the blend films was only 5 wt%, obvious antibacterial activity could also be observed. Compared to the pure medium and starch film, OD of the blend films was much lower. Furthermore, it was also found that the antibacterial activity of blend films enhanced by increasing the content of chitosan in blend systems.

4. Conclusion

Starch/chitosan blend films were prepared successfully by compression-molded starch-based mixture in physical gel state. The tensile strength and the flexibility of starch film were improved largely after incorporating 20% chitosan into starch film.

X-ray diffraction and SEM analyses of starch/chitosan blend films indicated that there was interaction and microphase separation between starch and chitosan molecules. After irradiation, there is no obvious change in the structure of starch/chitosan blend films, but antibacterial activity was induced due to the degradation of chitosan in blend films under the action of irradiation.

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