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Full Length Research Paper

Effect of γ-irradiation on the physicochemical properties of mixed soy protein isolate/starch material

Weihe Xu[1], Boye Liu[2], Hongshun Yang[2], Kunlun Liu[2], Suxian Jia[2] and Fusheng Chen[2]

[1]College of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450052, China.
[2]College of Food Science and Technology, Henan University of Technology, Zhengzhou 450052, China.

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To elucidate the effect of γ-irradiation on the molecular structure and mechanical properties of biodegradable material composed by soybean protein isolated (SPI) and starch, SPI and starch were mixed and modified by succinic anhydride and irradiation. Different doses (0 to 50 kGy) γ-irradiation on the elongation, tensile strength, viscosity, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and color of the mixed material were investigated. Gamma-irradiation decreased the viscosity of the material due to cleavage of the polypeptide chains of SPI. Irradiation treatment also increased the yellowness index of the mixed material. This study reveals that γ-irradiation can be applied as a useful cross-linking measure to improve properties of mixed SPI and starch, which provide a valuable chance to replace conventional petroleum-derived plastics, adding more value to this vast agricultural resource.

Key words: Soybean protein isolate, starch, biodegradable material, irradiation.

INTRODUCTION

Since the 20th century, research of synthesis of high polymer plastic progresses has advanced by leaps and bounds which brings the huge convenience for people's life (Derraik, 2002). However, with the growing production of plastic, it also brought lots of serious environmental pollution all over the world. For instance, groundwater and soil are seriously polluted; the safety of animals is also threatened (Lithner et al., 2011), landfill occupies land for a long time, and burning brings toxic gas which leads to further pollution (Lemieux et al., 2004).

The annual generation of plastic wastes in China amounts to 4 million tons with only 10% recovered and recycled (dropped from about 20% in 1980), 20 to 30% incinerated or landfilled and 60 to 70% dumped, littered and washed away (Ren, 2003). As a result of increasing concerns of environmental pollution caused by non-biodegradable petroleum-based plastics and exhausting of petroleum resources (Jan and Filip, 2009), researchers are interested in making natural degradable and environmentally-friendly plastics from renewable resources as alternatives to non-biodegradable petroleum-based plastics. Therefore, the search for new resources of sustainable development, exploration and research of environment-friendly materials become an important topic (Bordes et al., 2009).

In order to solve these problems, people have continued to carry out new explorations; the research of soy protein isolate/starch biodegradable materials has become a hot issue recently. As inexhaustible natural polymer materials, soy protein isolate/starch can be easily modified by physicochemical methods (Park et al., 2000; Liu et al., 2008). At the same time, they are biodegradable, biocompatible and safe, etc. Unfortunately, biodegradable materials made from soybean proteins have poor mechanical and water resistant properties, which are limitations for plastic materials (Gonzalez et al., 2011). Therefore, it is necessary to add filling agents to improve plastics processing properties, and some suitable modified methods and small molecule plasticizers (Tian et al., 2009; Kumar et al., 2009; Mo et al., 2002; Kalichevsky et al., 1993) can be used to improve the plastic's function. Soybean protein isolated (SPI) contains a great deal of hydrophilic groups, such as hydrogen bond, carboxyl, hydroxy and amino, etc. Due to its structural characteristics, aldehyde group of starch

*Corresponding author. E-mail: weihexu@haut.edu.cn. Tel: +86 371 67756837.

can be integrated with SPI, which is helpful to increase the mixture molecular mass with the resulting materials having better water resistance properties. These bio-materials made by the pure SPI are rigid, brittle and expensive. As a filling agent, starch enhances the material's process ability and reduces cost.

In past studies, soybean protein-based materials were prepared by injection molding (Otaigbe et al., 1999), compression molding (Mo and Sun, 1999; Ogale et al., 2000) and lamination (Ghanbarzadeh and Oromiehi, 2009). As a new technology, irradiation technique has its own strong points, such as, been suitable for low temperature processing, has good maneuverability, high purity and good quality, less energy consumption and it meets the future trend of environmental protection. γ -Irradiation affects soy proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds and formation of protein free radicals (Cheftel et al., 1985). Chemical changes of the proteins that are caused by γ-irradiation are fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis (Cho and Song, 2000). γ -Irradiation may generate free radicals on starch mole-cules and these free radicals are capable of hydrolyzing chemical bonds, thereby cleaving large molecules of starch into smaller fragments of dextrin (Grant and G'Appolonia, 1991). Through the irradiation technology, the SPI molecule combines with the starch molecule firmly in favor of increasing the material's capability and possessing a better exterior appearance.

The objective of this study was to investigate the effects of starch (a filling agent), plasticizer (water and glycerol) and irradiation on the properties of soybean protein isolate based biodegradable materials used for plastics, including tensile strength and elongation at break, color, Fourier transform infrared spectroscopy (FTIR) and viscosity of the material plasticized by complex plasticizer.

MATERIALS AND METHODS

Sample preparation

Powders of SPI (Shandong Wandefu Protein Co., Ltd.) and starch (Tianjin Kermel Co.,Ltd.) were mixed (94:6, 91:9, 88:12, 85:15, 82:18 w/w) and dispersed in epichlorohydrin (1:3, w/w), then 15% (w/w) succinic anhydride (Medicine group Chemical Co., Ltd.) was added and stirred for 0.5 h, respectively. The dispersion was vacuum filtrated and then air-dried. Plasticizers [1.5 g water, 1 g caprolactam (Tianjin Chemical Regent Co., Ltd.)] were added to the dried powders (10 g) in drops and mixed in a mixer. The mixture was equilibrated for 48 h to ensure an effective plasticization and even distribution of plasticizer. The mixture was then irradiated at 0, 10, 20, 30, 40 and 50 kGy at room temperature in air using a ^{60}Co gamma irradiator (cobalt radiation source 120000 Ci, Tongweisuo Research Institute Co., Ltd, Henan Academy of Sciences, China). The plasticized soybean protein powder was placed into a molder and compression-molded in a heat press at 15 MPa with 120°C for15 min.

Tensile testing and elongation at break

The molded soybean protein/starch materials were equilibrated at 23°C and 50% relative humidity for 48 h before testing. The specimen was then cooled to room temperature at the same pressure and was cut into a dog-bone shape according to standard method ASTM D638-91 (ASTM Standard, 1993). The tensile strength (TS), elongation at break (E) and strain at break were calculated using an instron universal testing machine (CMT6503, Shenzhen SANS Test Machine Co., Ltd, China) according to the standard test method ASTM D638-91. The crosshead speed was 5 mm/min, and five replicates were tested for each sample.

Color measurements

Colors were measured using a colorimeter (CR-400, Konica MinoltaCo., Ltd, Japan). Specimens (4 cm×4 cm) were placed on a white standard plate and the Hunter lab color was used to measure color: L = 0 (black) to L= 100 (white); a = _80 (greenness) to a = 100 (redness); and b = _80 (blueness) to b = 70 (yellowness). Total color difference (ΔE) was calculated from five measurements which were taken at different locations on each specimen (Lee et al.,2005).

$$\Delta E = [(L_{sample} - L_{standard})^2 + (a_{sample} - a_{standard})^2 + (b_{sample} - b_{standard})^2]^{0.5}$$

Measurement of viscosity

Viscosity of SPI solutions irradiated at various radiation doses was determined after 13 min mixing at 160~960 r/min at 25°C using a viscometer (RVA-4, New Port Co., Ltd, Australia).

FTIR

After samples were mixed with KBr, the mixture was then treated by the extrusion process (Souillac et al., 2002). FTIR spectra were collected with a QWF-510 FTIR spectrophotometer equipped with a standard DTGS detector. The spectra were recorded at the absorbance frequency from 4000 to 400 cm^{-1} mid infrared region at a resolution of 4 cm^{-1} with 128 co-added scans. At least triplicate spectra were recorded for each sample.

SEM

SEM was used to characterize the microstructure of SPI/starch material. SPI/starch materials were made conductive by sputter-coating with a gold-palladium alloy coater (Hitachi Co., Ltd, e-1010 ions putter, Japan). The coated materials were dried in a desiccator. Samples were then examined using a SEM (FEI Co., Ltd, Quanta 200, USA) at an accelerating electron voltage of 20 kV. Micrographs for sample surface were obtained at 800×magnifications.

RESULTS AND DISCUSSION

Effects of irradiation on properties of the mixed material

The effect of radiation dose on E and TS of soy protein isolate/starch biodegradable materials are showed in Figure 1. The E increased and TS decreased with the

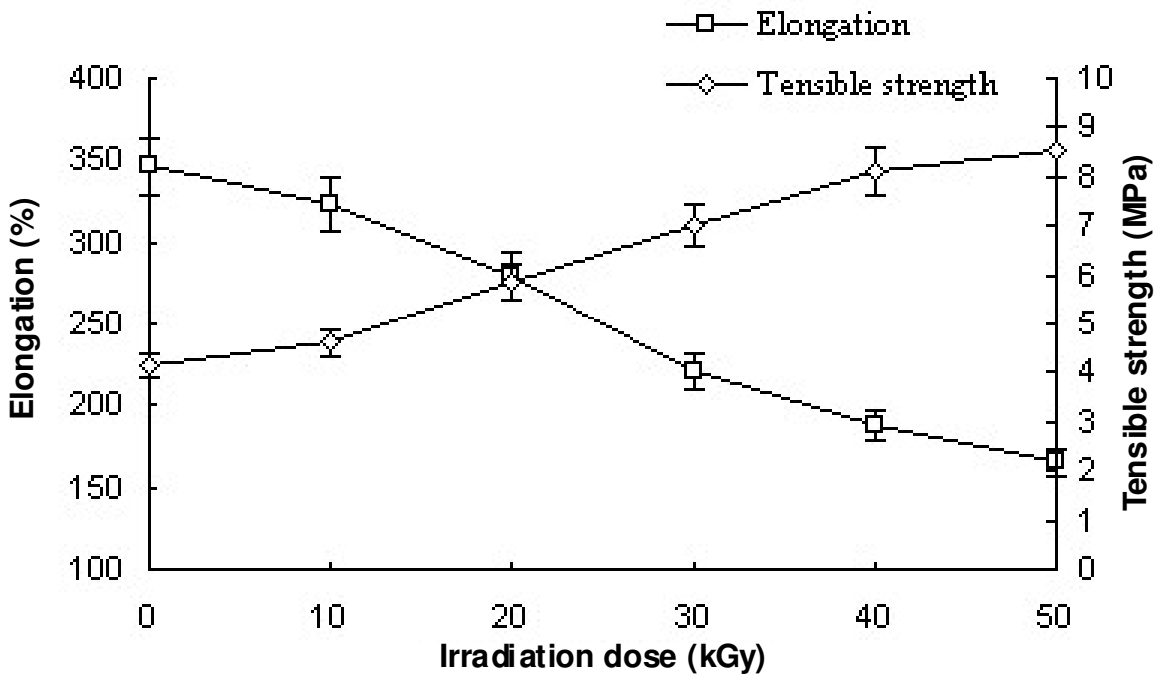

Figure 1. Effect of radiation dose on E and TS of SPI/starch (88:12) biodegradable materials.

increase of radiation dose within the range of 0 to 50 kGy. The reason was that irradiation produced polymer radicals. Those radicals resulted in the lost in the main chain of two hydrogen atoms; alkyl radicals reacted with each other to form H-crosslinked or reacted with free radical of the end of molecular chain to form T-corosslinked. After irradiation, water in samples produced excited state of oxidative free radicals that had oxygen-containing groups (carbonyl). It contributed to form amide link and improved the polymer surface polarity and the compatibility of them. At the same time, interface bonding strength was enhanced. The increase of the average molecular weight and partially increased polymer molecular weight could reduce the chance of cracking so that cross-linking increased the tensile strength (Kober and Gonzalez, 2007). Because the rate of forming free radicals was proportional to dose rate, increasing irradiation dose resulted in more cross-linking reaction. Therefore, with the increase in tensile strength, elongation at break was inversely deceased.

Effect of radiation dose on water absorption capacity (WAC) of SPI/starch biodegradable materials are showed in Figure 2. It shows that WAC decreased with the increase of radiation dose from 10 to 50 kGy. Radiation effects were obvious in the range of 10 to 30 kGy. Starch contains many hydrophilic groups. When it comes to the range of 40 to 50 kGy, WAC of samples remained much stable. Introducing cross-links to polymer increased chemical stability. It seems that γ-irradiation modified the conformation of proteins in the biomaterial mixture to a certain extent that had more ordered and more stable structures, and then, the swelling of the biomaterial in solvent decreased. It further decreased the possibility of deformation owing to water absorption capacity. Besides, irradiation caused the formation of c-c bond which made hydrolysis more difficult. Therefore, the water resistance was improved and the solubility decreased (Lacroix et al., 2002).

Effects of filling agent (starch) on properties of the mixed material

Effects of starch content on E and TS of SPI/starch biodegradable materials at 30 kGy are showed in Figure 3. E increased and TS decreased with increasing starch content within the range of 6 to 18%. The compound between starch and protein was not completed by a single function but a comprehensive result which was produced by the covalent bond, electrostatic interaction, van der Waals forces, hydrogen bonding, hydrophobic interactions, ionic bonds, the role of volume exclusion and molecular entanglement (Kruif and Tuinier, 2011). These functions existed in different fragments and side chains of two kinds of macromolecules and maintained the structure of compounds. The ε-NH_2 of protein molecules reacted with starch. They formed a protein-starch compound which caused the protein subunit dissociation to occur and the polypeptide chain extended fully. Spatial structure changes increased the molecular

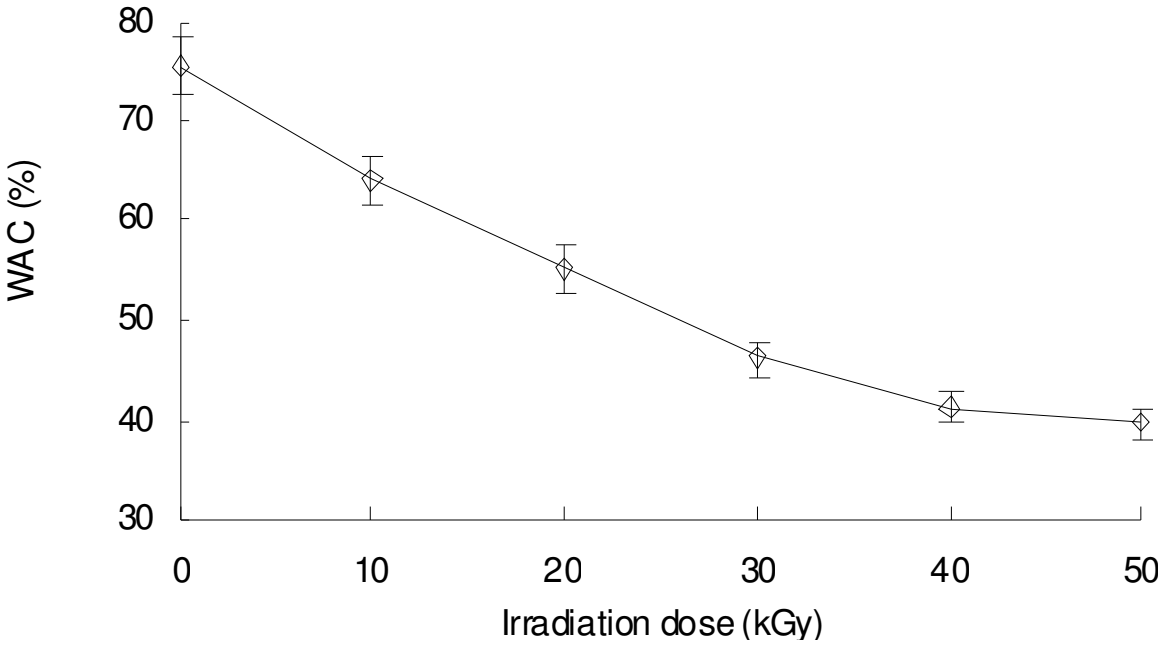

Figure 2. Effect of radiation dose on WAC of SPI/starch (88:12) biodegradable materials.

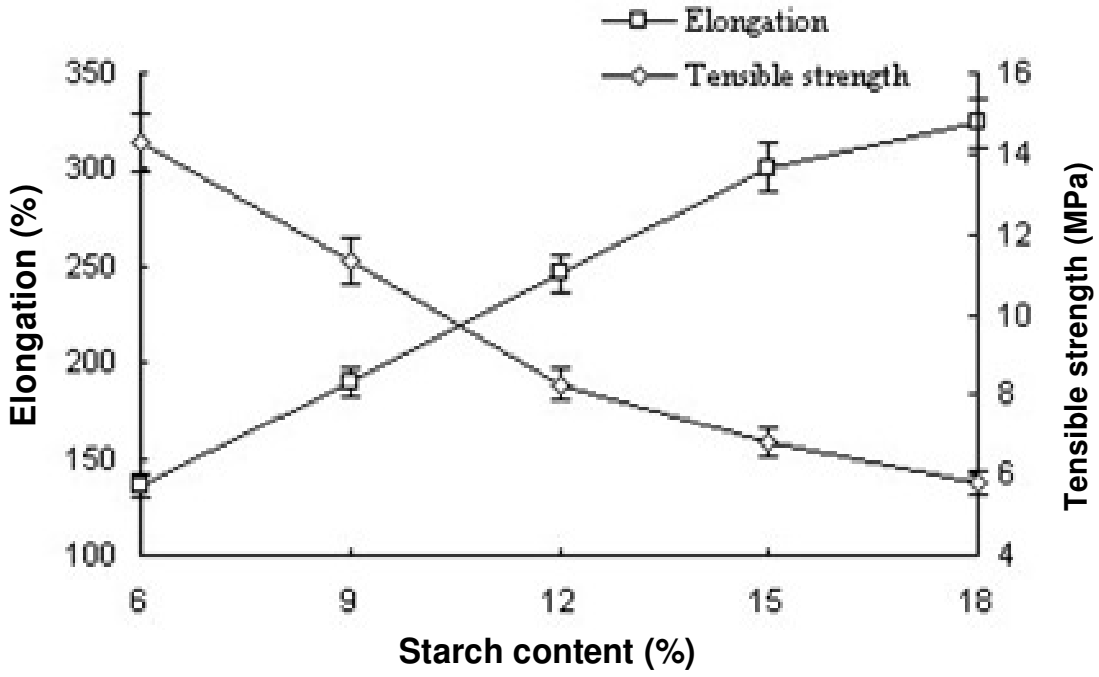

Figure 3. Effects of starch content on E and TS of SPI/starch biodegradable materials.

volume, improved flexibility and improved the material elongation. In addition, starch was cross-linked with protein amino acid residues that introduced exogenous moieties to protein molecules. It changed the distribution of protein molecular charge and resulted in the drop of surface charge of protein molecules. It also increased the molecular volume, improved flexibility and improved the material elongation.

Effects of starch content on WAC of soy protein isolate/ starch biodegradable materials at 30 kGy are shown in Figure 4. WAC slightly decreased with the increasing starch content within the range of 6 to 12%.

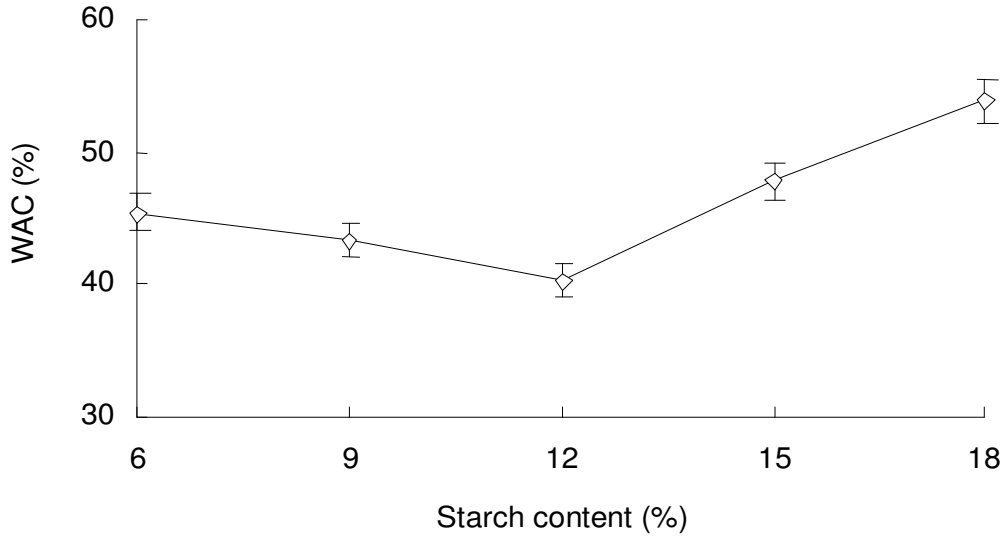

Figure 4. Effects of starch content on water absorption capacity of SPI/starch biodegradable materials.

Table 1. Effect of γ-irradiation on the color of SPI/starch biodegradable materials.

Irradiation dose(kGy)	L	a	b	ΔE
0	81.72±0.54^{a}	4.36±0.11^{a}	14.20±0.08^{d}	17.06±0.26^{a}
10	80.87±2.59^{a}	3.25±0.29^{b}	14.62±0.16^{cd}	16.73±0.20^{ab}
20	82.06±1.24^{a}	2.31±0.12^{c}	14.97±0.20^{c}	16.42±0.07^{b}
30	83.76±0.72^{a}	0.61±0.20^{d}	15.10±0.13^{bc}	15.82±0.11^{c}
40	84.44±0.87^{a}	0.06±0.05^{e}	15.49±0.14^{ab}	15.47±0.09^{c}
50	84.61±0.22^{a}	-0.06±0.06^{e}	15.96±0.24^{a}	15.42±0.23^{c}

Values are means of five replicates ± standard deviations. Values in the same column with different superscript letters indicate differences by multiple range test (P <0.05).

Increased starch content promoted cross-linking by irradiation of SPI/starch. It enhanced the effect of irradiation and decreased WAC (Salmoral et al., 2000). When the starch content was more than 12%, WAC increased with the increase of starch content. As the starch content increased, hydrophilic group was introduced, which added hydrophilic directly to SPI-starch compound, and the introduction of foreign groups also changed the conformation of proteins. Many hydrophilic groups were exposed, that easily formed hydrophilic network structure and improved hydrophilic ability of the materials. These results indicate that the optimum content of starch to biomaterials would result in forming a more compact structure and could help natural polymers to conquest the hydrophilic weakness (Kim et al., 2008).

The analysis of hunter color values

Table 1 shows the effect of γ-irradiation on the color of mixed materials. There were significant differences in Hunter b value of irradiated materials. Increasing irradiation dose increased Hunter b value. Hunter b value at 50 kGy was 15.96, compared with 14.20 of the control sample. It was evident that γ-irradiation treatment increased in imparting yellow color to film. L value was also significantly increased and Hunter a value descended with increase of radiation dose. These results were in good agreement with the previous report that yellowness of materials were increased by UV radiation (Gennadios et al., 1998), addition of dialdehyde starch (Rhim et al.,1998), sodium doecyl sulfate (SDS) treatment (Rhim et al., 2002) and heat curing (Kim et al., 2002).

Figure 5 shows effects of γ-irradiation on SPI/starch biodegradable material. It revealed that γ -irradiation treatment of SPI solutions also affected the viscosity of the mixed powder (Figure 5). When irradiation dose was under 30 kGy, non-covalent bonds and the disulfide bonds in SPI molecules were broken to cause subunit disaggregation and unfolded peptide chains, and even protein disaggregation and unfolding. Irradiation

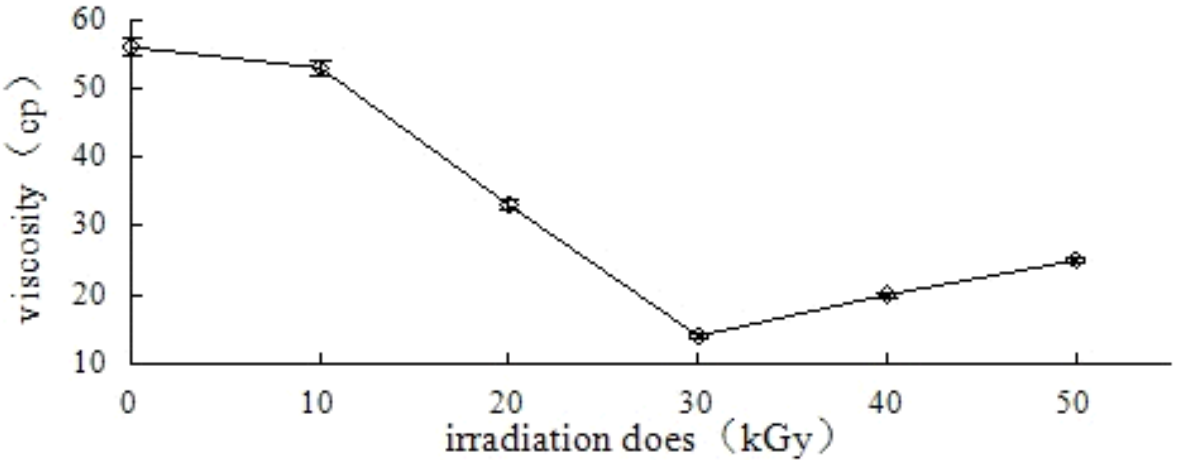

Figure 5. Effects of γ-irradiation on viscosity of SPI/starch biodegradable material.

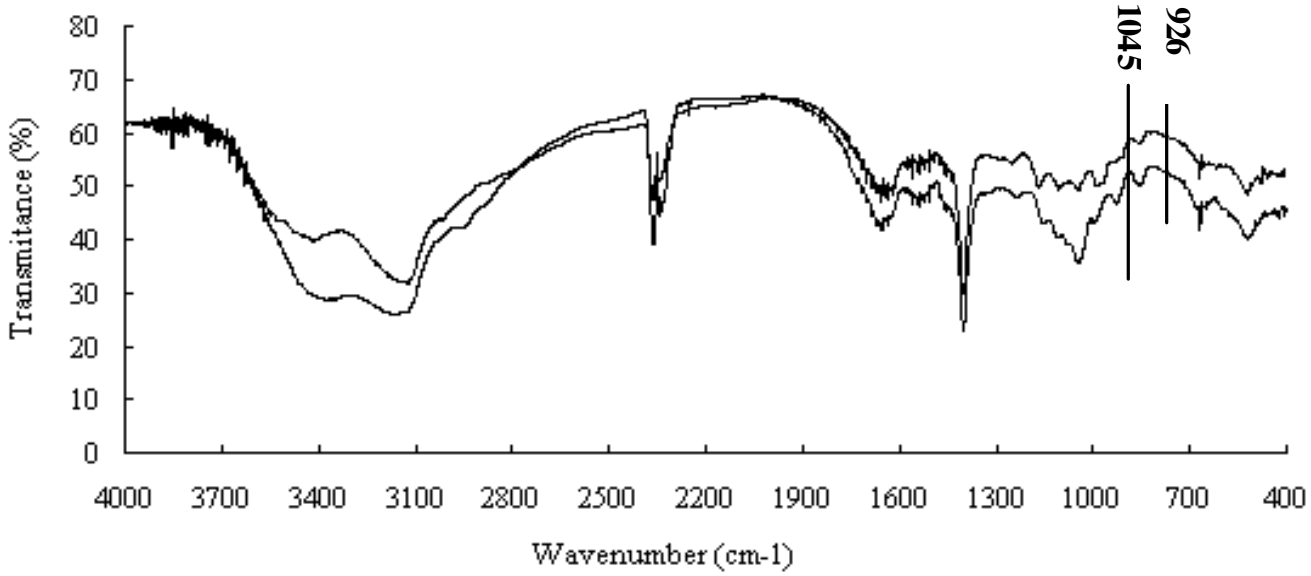

Figure 6. (A) FTIR spectra of SPI/starch biodegradable material without irradiation treatment and (B) FTIR spectra of modified SPI/starch biodegradable material by irradiation (50 kGy).

significantly decreased viscosity due to the conformational change of protein molecules by oxygen radicals generated by radiolysis of water. It was widely accepted that the reduction in starch viscosity was caused by the theory that irradiation apparently caused fissures or splitting in starch granules (Abu et al., 2006). Under high-power gamma-irradiation, viscosity value for 50 kGy was 14 cP, compared with 53 cP of the 10 kGg. Therefore, the probability of the effective collision between protein and starch molecules was increased. Moreover, the activation energy of graft reaction could be reduced significantly, thus, increasing the reaction selectivity, the reaction rates were increased significantly (Guan et al., 2011).

Figure 6 shows effects of y-irradiation on SPI/starch biodegradable material on the FTIR spectra. When protein molecule binds with saccharide molecule covalently, hydroxyl group will be added. The result was that there was a wide peak in the wavelength range of 3700~3200 cm^{-1} and an absorption in the wavelength range of 1260~1000 cm^{-1} appeared on the infrared spectroscopy. Compared with the infrared spectroscopy of unirradiated biodegradable material, the infrared spectroscopy of irradiated SPI/starch biodegradable materials have the creation of new chemical functions in the wavelength range of 3700~3200 cm^{-1} and 1260~1000 cm^{-1}. The absorption peak in the wavelength of 1045 cm^{-1} was the effect of bond C-N, which proved the addition of covalent bond. The absorption peak at 926 cm^{-1}, which

Table 2. Effect of γ-irradiation on secondary structure of protein.

Secondary structure	Unirradiated spi\starch material	SPI/starch material irradiated at 50 kGy
α-Helix	15.22±0.12[d]	1.02±0.13[d]
β-Pleated	38.03±0.16[a]	39.01±0.15[b]
β-Turn	26.37±0.10[b]	58.38±0.08[a]
Disordered structure	20.38±0.11[c]	1.56±0.09[c]

belongs to the wavelength range of 905~961 cm^{-1}, was the bending vibration of the external plane of C-H double stranded. The infrared spectroscopy of irradiated SPI/ starch biodegradable materials have the creation of new chemical functions in the wavelength of 926 and 1045 cm^{-1} that proved the complex reaction of SPI and starch that were added.

Acid amides I band (1600 to1700cm^{-1}) is the most valuable band region for the investigation of the second structure of protein conformation (Byler and Susi, 1986). Infrared spectroscopy is a reliable method for the investigations that determines the second structure of protein according to the stretching vibration v(C-C) of C-C bond.

Table 2 shows effects of y-irradiation on secondary atructure of protein. During the analysis of secondary structure of protein, as the increase of irradiation dose, amylase and amylo-pectin were broken and they had cross linking reaction with protein. α-Helix and β-pleated sheet structure were usually buried in the interior of a polypeptide chain. α-Helix was first formed during the establishment of the formation of globulin conformation. Compared with β-pleated sheet, α-helix was more unstable and was transformed into β-pleated sheet. So, α-helix greatly reduced under irradiation show polypeptide chains recombination in the interior of protein. The tensile strength and water resistance of biodegradable material closely related with the orderliness of molecular structure. Disordered structure greatly reduced after irradiation, which was consistent to the increase of tensile strength. This reduction also increased the degree of the smoothness of the material surface and reduced jut and gap.

SEM

Microstructure observed by scanning electron micro-scope showed that irradiated SPI/starch materials had glossier and smoother surface than the control material (Figure 7). Due to the good repeatability of the samples, the results were the same every time. The image displayed the consistency on the different observation point of the sample. Apart from tiny spots, the surface of SPI materials was unobvious rough and uneven. When unirradiated SPI/starch material with SPI/starch material irradiated was compared at 50 kGy, it was evident that the former had some fissure and lump. It was possible that irradiation treatment enhanced cross-linking density of the material and reduced non-uniformity of raw materials, thereby causing the surface of the material to become more smooth which is significant for strengthening water resistance capability. The result is in good agreement with a previous report (Vachon et al., 2000) that the microstructure of protein films that were cast from irradiated film forming solutions was smoother than that of the control film.

Conclusion

This study clearly testifies that starch granules would be modified by γ -irradiation. γ -Irradiation treatment of the SPI caused the disruption of the ordered structure of the protein molecules as well as cleavage of the polypeptide chains of SPI, such as, reduced α-helix and enhanced β-turn. Accompanied by the occurrence of the degradation, the cross-linking increased with the increasing dose. Therefore, the viscosity did not reduce much further above 30 kGy because of cross-linking of the proteins/ starch and aggregation of the polypeptide chains, resulting in change of water resistance capability and TS, and formation of more glossy and smoother surface.

Starch is able to integrate with SPI, which is helpful to increase the mixture molecular mass with the resulting materials having better water resistance properties. These biomaterials made by the pure SPI are rigid, brittle and expensive. As a filling agent, starch enhances the material's process ability and reduces cost. These effects may increase the applicability of such materials in biodegradable packaging or other industrial applications. As a result, γ-irradiation can be a useful tool (as a cross-linking agent) to improve mixed properties of SPI and starch; which offers a new method using the composite raw materials to replace conventional petroleum-derived plastics, adding new function field of the vast agricultural resource.

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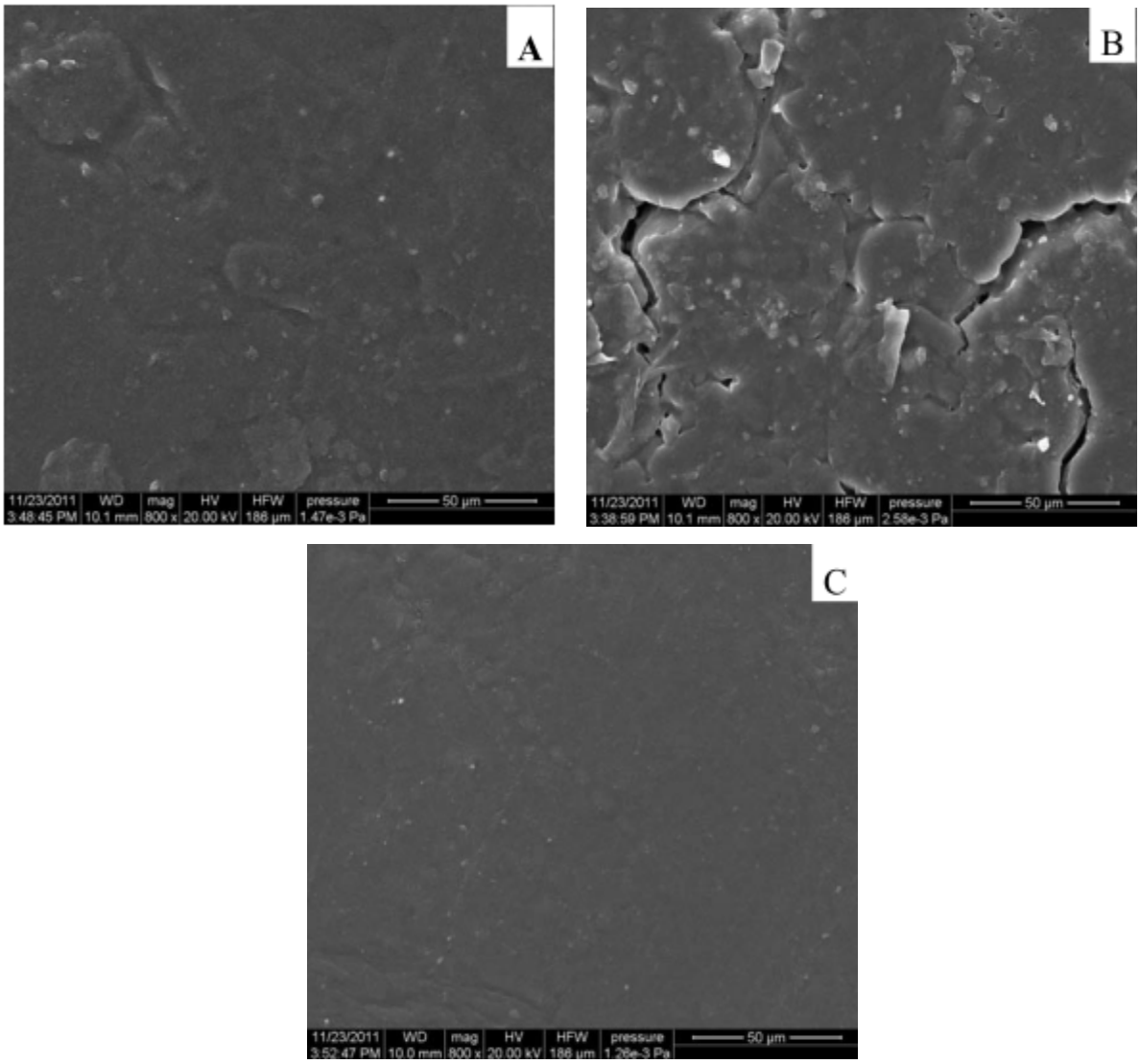

Figure 7. Microstructure of irradiated SPI films (800×magnification). A, unirradiated SPI material; B, unirradiated SPI/starch material; C, SPI/starch material irradiated at 50 kGy.

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