

Preparation and Characterization of Maillard Reacted Chitosan Films with Hemicellulose Model Compounds

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ABSTRACT: The Maillard reaction between chitosan and hemicellulose model compounds such as mannose, xylose, and glucuronic acid was investigated. Chitosan and the compounds were dissolved in 1% acetic acid and were dried at 50°C. In an analysis of the film obtained, weight increase, color difference, FTIR, relative amount of free amino groups, insolubility to dilute acetic acid, and tensile properties were measured. The film weight increased gradually as the compounds were added, and the color difference changed significantly. In addition, the relative amount of free amino groups decreased rapidly, and the insoluble matter increased markedly. In particular, xylose

brought about drastic changes in a small amount addition. According to the results of FTIR spectra, the cleavage of sugar unit of chitosan and the formation of heterocyclic compounds were observed in all compounds. The formation of carbonyl and carboxyl groups was observed with the addition of a large amount of the compounds. The tensile strength was improved in 10–20 wt % additions of the compounds. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 2481–2487, 2008

Key words: chitosan; Maillard reaction; mannose; xylose; glucuronic acid

INTRODUCTION

Chitosan is a deacetylated derivative of chitin that is extracted mainly from crab and shrimp shells. Chitin is widely distributed in nature and is abundant biopolymer. In terms of its general properties, chitosan is a cationic polysaccharide with amino groups and is soluble in dilute acid but insoluble in water. A great deal of research has been performed regarding the utilization of chitosan as a functional polymer.¹ In particular, research on the interaction of chitosan with wood or wood components, which are very useful biomass resources, has become of interest in recent years.^{2–10} In our previous report, the interaction of chitosan with cellulose model compounds based on the Maillard reaction was investigated.¹¹ It was clarified that chitosan was markedly modified by increasing the amount of the compounds added.

Generally, the Maillard reaction brings the browning of compounds due to interactions between carbonyl groups such as reducing sugars and amino compounds such as amines, amino acids, peptides, or proteins. It is well known that this reaction occurs

during the heating, storage, and processing of foods. Therefore, a number of studies have been done on the Maillard reaction in food.^{12–15} On the other hand, studies on the Maillard reaction between chitosan and wood components are scarcely performed. When considering the further application of chitosan to wood and wood components in the future, it is important to clarify the basic characteristics of the Maillard reacted chitosan with reducing sugars that are related to wood components other than cellulose components. In this study, mannose, xylose, and glucuronic acid, which are hemicellulose model compounds, were used. Some of the chemical and physical properties of the chitosan film that reacted with the model compounds were investigated.

MATERIALS AND METHODS

Materials

Purified chitosan powder obtained from Kimica (Tokyo, Japan) was used without further purification. The degree of deacetylation and the molecular weight were about 85.6% (colloid titration method) and 35,000 [viscosity method (M_v)], respectively. D-Xylose and D-glucuronic acid were obtained from Sigma-Aldrich Japan (Tokyo, Japan). D-Mannose was purchased from Wako Pure Chemical Industries, (Osaka, Japan). All materials were previously vacuum-dried at 60°C for 24 h. Acetic acid, N/400 potassium polyvinylsulfate, and 0.1% toluidine blue

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indicator solution were also purchased from Wako Pure Chemical Industries.

Film preparation

Chitosan (0.50 g) was exactly dissolved in 24 g of 1 wt % acetic acid solution, and the model compounds were individually added to the chitosan solutions. The weight ratios of chitosan and the model compound were adjusted exactly to 10 : 0, 9 : 1, 8 : 2, 5 : 5, and 3 : 7. The solutions were stirred for 30 min at room temperature and then were filtered through filter paper. After the removal of air bubbles under reduced pressure, the solutions were poured into a small plastic tray (5 cm × 8 cm) and were dried in an oven at 50°C for about 20 h. The films obtained were immersed in an ethanol/4% sodium hydroxide mixture (7 : 3, w/w) for 1 h to remove residual acetic acid and were washed thoroughly with an ethanol/distilled water mixture (7 : 3, w/w) to remove alkali and residual sugar. After the washings showed a neutral pH, the films were dried at an ambient temperature for 30 min. Finally, the films were vacuum-dried at 50°C for 15 h and stored in a desiccator with silica gel.

Weight increase

The weight increase was determined according to the following equation:

$$\text{Weight increase (\%)} = (W_{c+m} - W_c) \times 100 / W_c$$

where W_{c+m} is the weight of the compound-added chitosan film and W_c is the weight of the pure chitosan film. The experiment was performed in triplicate, and average value with standard deviation was calculated.

Measurement of color

The films were conditioned at 20°C and RH65% for more than 48 h prior to color measurement, and were set on five-layered filter paper sheets. The color difference of the film was evaluated on the basis of the pure chitosan film. The film color was measured using a colorimeter (Gardner color-guide 45/0, Toyo Seiki Seisaku-sho Tokyo, Japan) according to the CIELab color system. The sensor head was 20 mm in diameter, and the measurement was performed under a D65 light source and an angle of 10°. Three positions of each film were tested and the average value was used to evaluate the film color. The CIE-Lab color parameters (L^* , a^* , b^*) were used to calculate the color change. The differences in the lightness (ΔL^*) and color (ΔE^*) were calculated using the following formulas.

$$\Delta L^* = L_t^* - L_r^*$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

where L_t^* is the lightness of compound-added chitosan film, L_r^* is pure chitosan film as a control reference, and Δa^* and Δb^* are the chroma differences based on pure chitosan film.

FTIR measurement

All of the infrared spectra of the films were obtained with an FTIR620 spectrometer (JASCO International Co.) by using the KBr pellet method and were recorded by means of an average of 64 scans at a resolution of 4 cm⁻¹.

Free amino group and insoluble matter

The amount of free amino groups in the film was determined by the colloidal titration method. Each film (0.20 g) was dissolved in 99.80 g of 5 wt % acetic acid solution for 3 h at room temperature, and the insoluble matter was filtrated by a glass filter. A total of 1.00 g of the filtrate was mixed with 30 mL distilled water. After 2–3 drops of 0.1% toluidine blue indicator were added, the filtrate was titrated against N/400 potassium polyvinylsulfate. The weight of free amino group in the solution was obtained, and relative amount of free amino groups in the film was calculated based on the value of pure chitosan film. On the other hand, the insoluble matter was washed with distilled water and was vacuum-dried at 50°C for 15 h. The weight was measured, and FTIR analysis was performed using the KBr pellet method. The titration and the measurement of insoluble matter were carried out in triplicate.

Tensile test

First, dried films were conditioned at 20°C, RH 60% for more than a week, and rectangular specimens (40 mm × 8 mm) were cut out from the film using a paper cutter. Tensile test was performed using ORIEN-TEC STA-1150. About 15 specimens were tested for each condition. The specimens fractured at near to grip were excluded, and the 10 specimens out of 15 were adopted as the data. Initial grip separation and cross-head speed were 30 mm and 2 mm/min, respectively.

RESULTS AND DISCUSSION

Weight and color changes

Figure 1 shows the weight increase of each compound-added chitosan film. Regardless of the com-

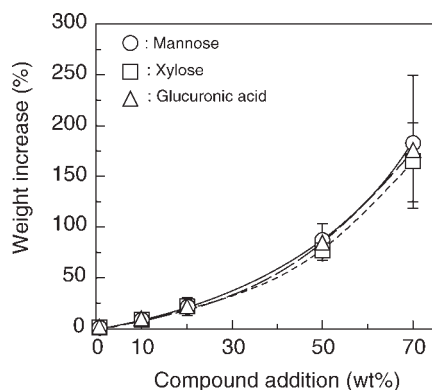


Figure 1 Weight increase of the compound-added chitosan films.

pounds, the weight increased similarly up to 70 wt % addition. In the case of 70 wt % addition, the average weight increases detected for mannose, xylose, and glucuronic acid were 184%, 164%, and 176%, respectively. This indicates that each compound was significantly incorporated into chitosan as the addition amount increased.

Figure 2 shows the color change of each chitosan film to which the compounds were added. The ΔL^* value of mannose-added chitosan decreased up to the 50 wt % addition, and then remained at a similar value. When xylose and glucuronic acid were added, the ΔL^* values decreased linearly with the addition

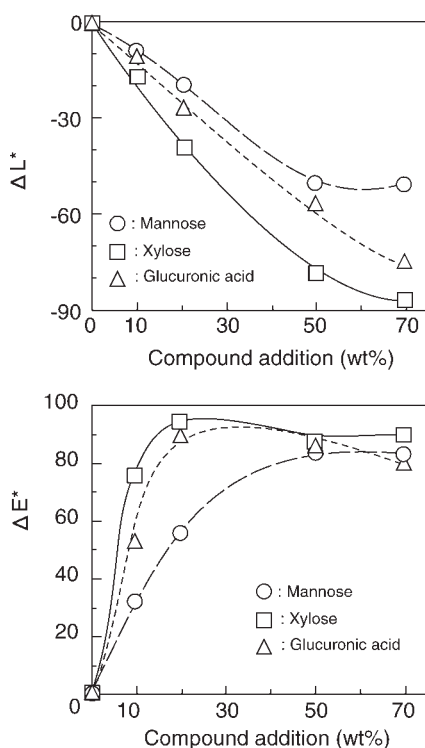


Figure 2 Changes in color (ΔE^*) and brightness (ΔL^*) differences of the compound-added chitosan films.

amounts. In particular, the addition of xylose brought a lowering of the lightness considerably. The ΔE^* value of xylose-added chitosan increased remarkably up to the addition of 20 wt %. Similar behavior was observed in glucuronic acid-added chitosan. When mannose was added to chitosan, the value increased gradually. In the case of the 50 and 70 wt % additions, similar values were detected in all compounds. In any case, the color of the film changed to dark brown, indicating that the Maillard reaction was proceeding.

Changes in the chemical structure

The chemical structure of each film was characterized by using FTIR measurement. Figure 3 shows the infrared spectral changes of mannose-added chitosan films. In the IR measurement of chitosan, the assignments of representative peaks have been already clarified. The characteristic absorption bands between 4000 and 2500 cm^{-1} are 3480–3440 cm^{-1} , 3260–3270 cm^{-1} , and 2960–2878 cm^{-1} for the OH, NH, and CH stretching regions, respectively.¹⁶ The adsorption bands at around 1650 cm^{-1} , around 1560 cm^{-1} , and around 1310 cm^{-1} are assigned to Amide I,^{16,17} Amide II,^{16,17} and Amide III,¹⁷ respectively. In this study, the absorption peak at 1594 cm^{-1} that is attributed to the primary amino group was observed. The adsorption bands at around 1154 and 897 cm^{-1} are assigned to the C–O stretching and the β -D-configuration, respectively.^{16,18,19} When mannose was added to chitosan, the absorption peaks at

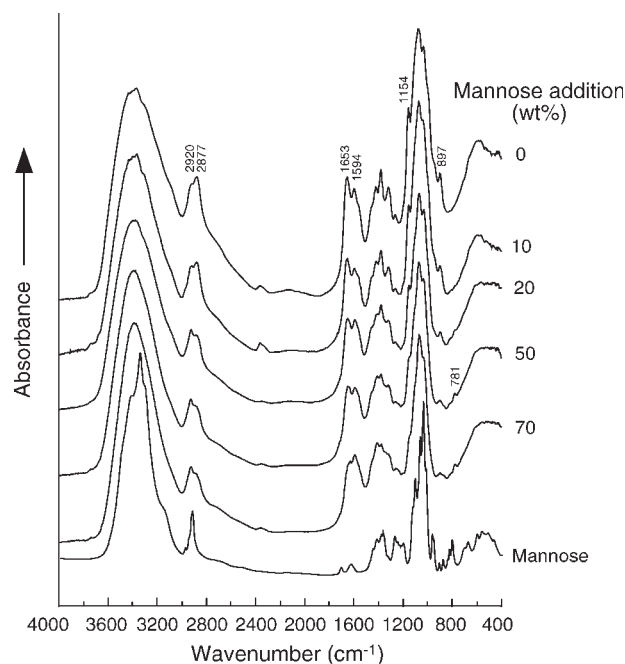


Figure 3 Infrared spectra of mannose-added chitosan films.

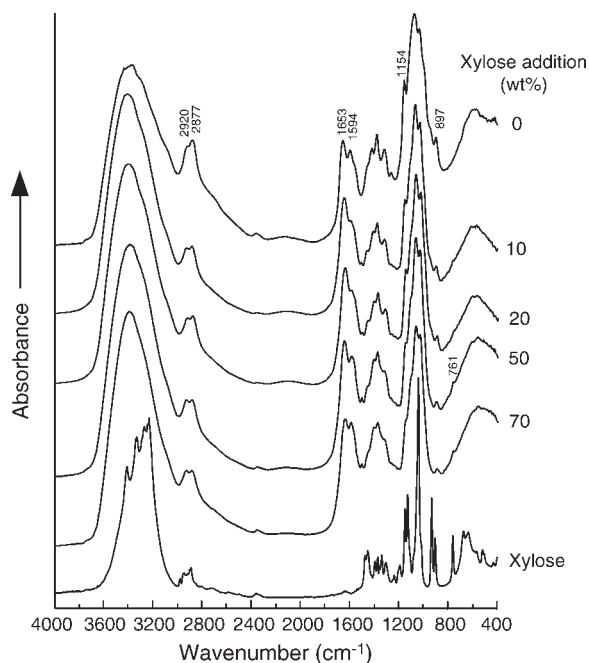


Figure 4 Infrared spectra of xylose-added chitosan films.

1154 and 897 cm^{-1} gradually decreased. This indicated that cleavage of the sugar unit of chitosan occurred. The absorption peak at around 1653 cm^{-1} gradually decreased and shifted to 1633 cm^{-1} . In the case of the 50% and 70% additions, the absorption band at around 781 cm^{-1} appeared as a small peak. It is known that the Maillard reaction forms furfural and its derivative, both of which have a carbonyl group, under a pH of 7 or below.¹⁴ In this study, the reaction system was in an acidic condition because it contained 1 wt % acetic acid. The absorption band at 700–800 cm^{-1} is characteristic of five-membered heterocyclic compounds such as pyrrole and furan.^{20,21} Therefore, the appearance of the absorption band at around 781 cm^{-1} was attributed to the formation of heterocyclic compounds.

Figure 4 shows the infrared spectral changes of xylose-added chitosan films. As with the mannose-added chitosan film, the absorption peaks at 1154 and 897 cm^{-1} decreased gradually, showing the cleavage of the sugar unit of chitosan. The absorption peak at around 1653 cm^{-1} shifted to 1645 cm^{-1} . The peak at 761 cm^{-1} appeared as a small peak in the 50 and 70 wt % additions. This absorption peak also seemed to be due to the formation of heterocyclic compound derived from the Maillard reaction.

Figure 5 shows the infrared spectral changes of glucuronic acid-added chitosan films. In this case, the peaks at 1154 and 897 cm^{-1} decreased gradually with increasing the glucuronic acid content. As a characteristic change, the peaks at around 1604 and 1414 cm^{-1} attributed to carboxylate²² increased

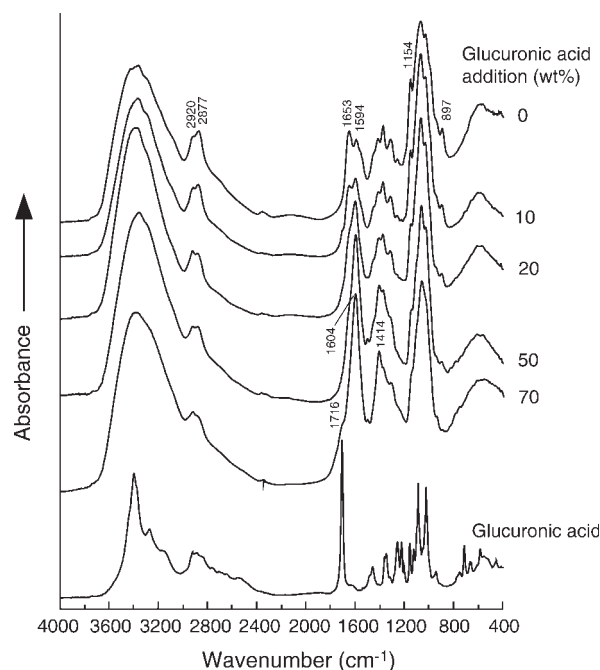


Figure 5 Infrared spectra of glucuronic acid-added chitosan films.

markedly. In the 70 wt % addition, the band at 1716 cm^{-1} appeared as a shoulder. Considering the absorption peak of glucuronic acid itself, the peak would be attributed to the carboxyl group of glucuronic acid.

As amino groups are consumed during the Maillard reaction, the change of the relative amount of free amino groups in each film was observed by the colloidal titration method. The results are shown in Figure 6. As a whole, the free amino groups decreased rapidly as the compound addition increased. In particular, when xylose was added at a proportion of more than 10 wt %, the value was less than 10%. This means that xylose, having a low molecular weight, reacted with chitosan easily. In the case of mannose and glucuronic acid, the addition of

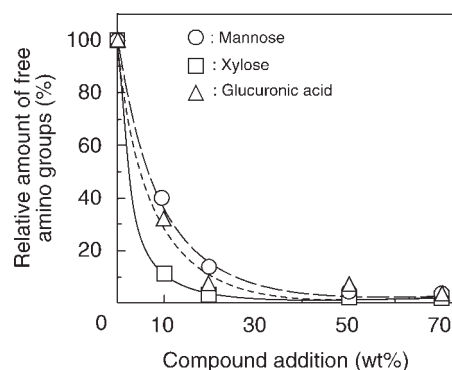


Figure 6 Free amino groups of the compound-added chitosan films.

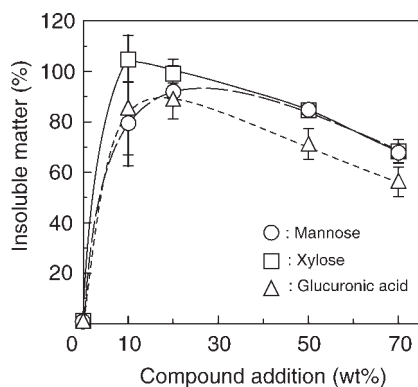


Figure 7 Insoluble matter of the compound-added chitosan films in 5% acetic acid solution.

20 wt % and more resulted in a value of less than 20%. It was clarified that the amount of free amino groups of chitosan decreased markedly with the addition of a small amount of the compounds.

Insoluble matter and chemical structure

The insoluble matter of each chitosan film in 5% acetic acid solution was determined by the colloidal titration method. The results were shown in Figure 7. The insoluble matter increased rapidly and then decreased gradually regardless of the compound. Generally, chitosan is soluble in a dilute acid to form the salt. However, it was demonstrated that chitosan changed easily to insoluble matter by the addition of the model compounds. In particular, 10 wt % addition of xylose brought almost complete insoluble matter. The results shown in Figure 6 suggest that the increase of the insoluble matter was related to the decrease in free amino groups. This indicates that the amino groups in chitosan would be markedly consumed by the Maillard reaction. The decrease of the insoluble matter at higher addition of the compounds would be due to dilute acetic acid-soluble substances formed by the Maillard reaction.

Figure 8 shows the infrared spectra of the insoluble matter of mannose-added chitosan films. The absorption bands at 1644 and 1558 cm^{-1} were clearly observed at the 10 wt % addition. The peak at 1558 cm^{-1} decreased gradually as the addition increased. As mentioned in Figure 3, the adsorption bands at around 1650 and 1595 cm^{-1} were attributed to C=O (Amide I) and the amino group, respectively. However, considering the results of Figures 6 and 7, the formation of Maillard reaction products via the Schiff base was expected. The absorption band at around 1650 cm^{-1} had been assigned to the C=N linkage derived from the Schiff base.^{19,23} Therefore, the absorption band at 1644 cm^{-1} would represent the overlap of the C=O group and the C=N link-

age. On the other hand, the amino groups of chitosan are consumed during the Maillard reaction and are converted to the secondary and tertiary amines gradually. Based on the results shown in Figure 6, the absorption band at 1558 cm^{-1} was mainly attributed to the secondary amine, and the decreases of the peak seemed to be due to the formation of tertiary amines. The absorption bands at 1152 and 899 cm^{-1} decreased gradually, indicating that the sugar unit of chitosan decreased in insoluble matter. The absorption band at 780 cm^{-1} attributed to heterocyclic compounds appeared gradually as a small peak, and the band at 1723 cm^{-1} attributed to carbonyl groups such as aldehyde and ketone appeared as a small shoulder at the 70 wt % addition.

Figure 9 shows the infrared spectra of the insoluble matter of xylose-added chitosan films. As with the mannose, the absorption bands at 1558 cm^{-1} attributed to secondary amine and 1152 cm^{-1} attributed to the C—O stretching decreased gradually as the xylose addition increased. The absorption band at around 898 cm^{-1} exhibited very small peak and lowered slightly. These findings suggested that secondary amines changed to tertiary amines and that the number of sugar units of chitosan decreased. The appearance of the small peak at around 761 cm^{-1} was observed at additions of 20 wt % and more, showing the formation of heterocyclic compounds. In addition, double small shoulders at around 1700 and 1765 cm^{-1} were observed. Each peak implied the formation of the carbonyl groups and carboxyl groups, respectively.

Figure 10 shows the infrared spectra of the insoluble matter of glucuronic acid-added chitosan films.

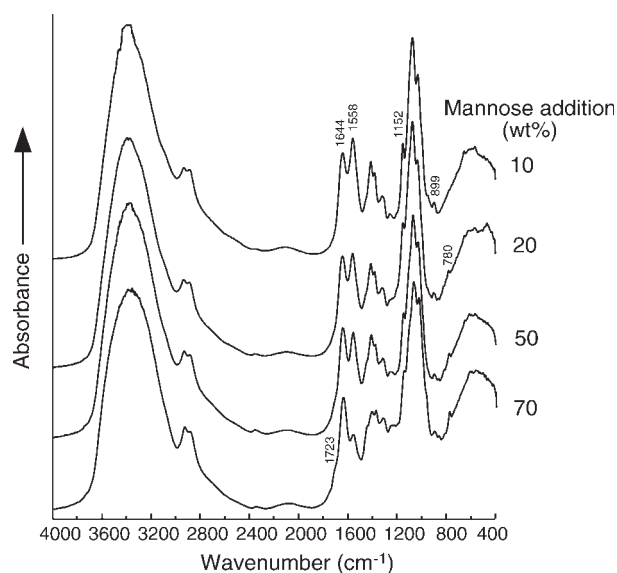


Figure 8 Infrared spectra of insoluble matter of mannose-added chitosan films in 5% acetic acid solution.

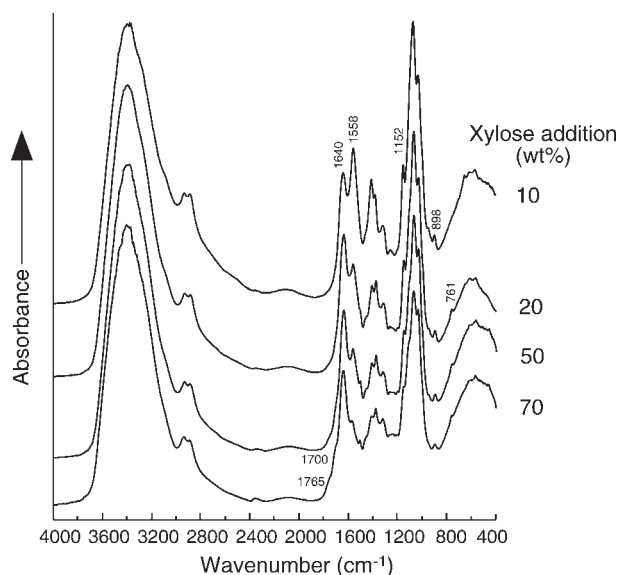


Figure 9 Infrared spectra of insoluble matter of xylose-added chitosan films in 5% acetic acid solution.

In the 10 wt % addition, the peaks at 1639 and 1559 cm^{-1} were observed clearly. However, the peak at 1639 cm^{-1} shifted to 1621 cm^{-1} , and a peak appeared at 1597 cm^{-1} instead of 1559 cm^{-1} as the addition was increased. The broad peak at 1600 to 1620 cm^{-1} seemed to be derived from the C=O group, C=N linkage, and carboxylate. The peaks at 1152 and 895 cm^{-1} lowered gradually, and the absorption bands at 768 and 1762 cm^{-1} appeared as a shoulder at the 70 wt % addition. These spectral changes indicated that the decrease of sugar units of chitosan, the formation of heterocyclic compounds and carboxyl groups took place in insoluble matter.

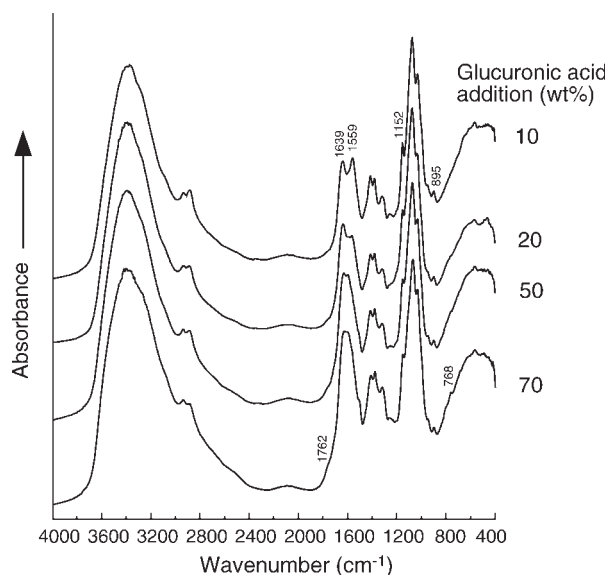


Figure 10 Infrared spectra of insoluble matter of glucuronic acid-added chitosan films in 5% acetic acid solution.

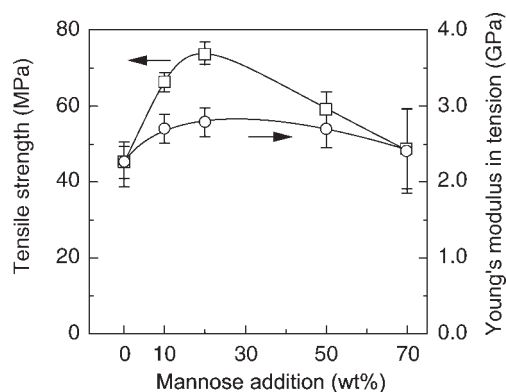


Figure 11 Tensile properties of mannose-added chitosan films.

Tensile strength

To investigate the mechanical properties of each film, tensile strength test was performed. Figure 11 shows the effects of mannose on the tensile properties of chitosan film. The tensile strength and the Young's modulus of pure chitosan film were 44.7 MPa and 2.26 GPa, respectively. The tensile strength increased up to the addition of 20 wt %, and then decreased gradually. The average value in 20 wt % addition was 74.0 MPa. Compared to pure chitosan film, the tensile strength was improved about 65%. In Young's modulus, the value increased slightly and then decreased. The maximum average value recorded was 2.78 GPa in 20 wt % addition. Figure 12 shows the effect of xylose on the tensile properties. The tensile strength was improved in the addition of 10 and 20 wt %, and then lowered slightly. The maximum average value was 60.2 MPa in 10 wt % addition. In Young's modulus, the maximum value was recorded in 10 wt % addition, which was 2.59 GPa. However, marked change was not recognized regardless of the addition amount. Figure 13 shows the tensile properties of glucuronic acid-added film. As with the other compounds, the tensile strength increased up to the addition of

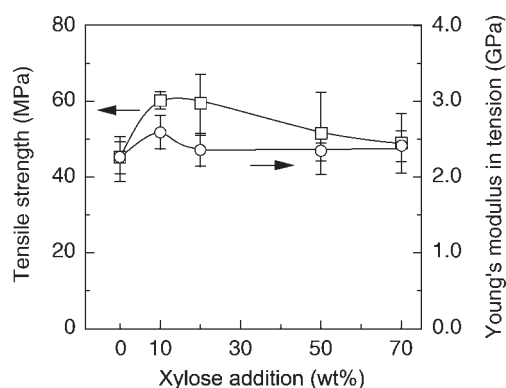


Figure 12 Tensile properties of xylose-added chitosan films.

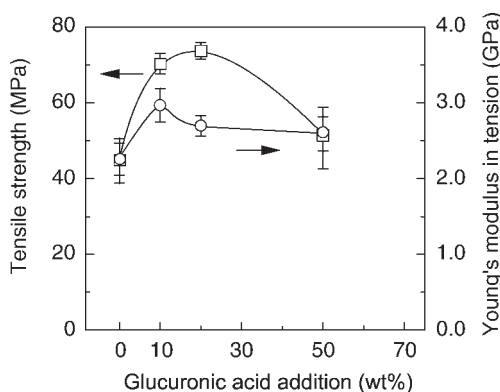


Figure 13 Tensile properties of glucuronic acid-added chitosan films.

20 wt %, and then decreased. In particular, the film of 70 wt % addition was too brittle to measure the tensile properties. The average value in 20 wt % addition was 73.8 MPa. The Young's modulus was improved a little by the addition. The maximum value was recorded in 10 wt % addition, which was 2.97 GPa.

It was made clear that a small amount addition of the compounds brought about the improvement of the tensile strength of chitosan. In particular, 20 wt % addition of mannose was the most effective. However, effect of the addition for the Young's modulus was small. As a whole tendency, the sugar units of chitosan existed clearly until 20 wt % addition of the compounds as shown in the results of FTIR. In this case, the Maillard reaction proceeded reasonably, and the molecular weight of the film would increase, resulting improvement of tensile properties. When the compound was added further more, the molecular weight would decrease with the progress of the reaction accompanying the cleavage in sugar unit and the formation of heterocyclic compounds. Therefore, tensile properties would lower.

CONCLUSIONS

Some characteristics of the Maillard reaction products obtained from chitosan and hemicellulose model compounds were investigated. When these model compounds were added to chitosan, the weights of the film obtained increased. Under the 70 wt % addition of the compounds, the weight increases recorded were more than 100%, regardless of the compounds. The film colors changed markedly as the addition of the compounds increased. The FTIR spectra of the film obtained showed that

the cleavage of the sugar units of chitosan and the formation of the heterocyclic compounds became more evident as the amount of added compounds was increased. The free amino group of the chitosan decreased rapidly as the compound was added, with the value being only a few percent at a 20 wt % addition or above. In addition, the films came to exhibit insolubility in 5% acetic acid. In particular, the addition of a small amount of xylose was effective for the insolubility. The FTIR spectra of insoluble matters showed that the formation of the Schiff base and the tertiary amine occurred. The cleavage in the sugar units and the formation of heterocyclic compounds, carbonyl, and carboxyl groups were also observed at high addition amounts. The 20 wt % addition of the compounds, especially mannose, was effective for the improvement of the tensile strength.

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