

Modification of chitosan by the Maillard reaction using cellulose model compounds

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Abstract

The Maillard reaction between chitosan and cellulose model compounds such as glucose and cellobiose was investigated. Chitosan and the compounds were dissolved in 1% acetic acid, and were dried at 50 °C. As the analysis of the film products, weight increase, color change, FT-IR, relative amounts of free amino groups, insolubility to dilute acetic acid and tensile properties were measured. When the compounds were added to chitosan, the film weight increased gradually and the color difference changed significantly. In addition, the relative amounts of free amino groups decreased rapidly, and the insoluble matter increased. The good tensile properties were obtained when the compounds were added at 20wt%. According to the results of FT-IR, the cleavages in sugar units of chitosan and the formation of heterocyclic compounds were observed. It was recognized that glucose reacts easily with chitosan compared to cellobiose. The progress of the Maillard reaction was dependent on the amount of the compound added.

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1. Introduction

Chitosan is a deacetylated product of chitin that is extracted from crab and shrimp shells. Recently, chitosan has received attention as a promising biomass resource, and it has been studied extensively with regard to its industrial applications (Majeti & Ravi, 2000). As the chemical structure has an amino group, chitosan exhibits a polycationic nature. On the other hand, wood is also the most abundant and available biomass resource, and shows generally polyanionic nature. Therefore, they are expected to cause a certain intermolecular interaction and affinity. In fact, many studies have been reported on the combination of chitosan and wood (Hirabayashi, 1989) or wood components (Hosokawa, Nishiyama, Yoshihara, Kubo, & Terabe, 1991; Hosokawa, Nishiyama, Yoshihara, &

Kubo, 1990; Hasegawa, Isogai, Onabe, Usuda, & Atalla, 1992; Hasegawa, Isogai, Onabe, & Usuda, 1992; Isogai & Atalla, 1992; Liu, Nishi, Tokura, & Sakairi, 2001; Mucha & Miśkiewicz, 2000; Rogovina, Akopova, Vikhoreva, Gorbacheva, & Zelenetskii, 2000). In our previous reports, the applicability of chitosan to natural wood adhesive was investigated (Umemura, Inoue, & Kawai, 2003; Umemura, Iijima, & Kawai, 2005).

Generally, the Maillard reaction brings the browning of compounds due to the interactions between carbonyl group 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; it influences the food quality by affecting factors such as color, flavor, taste, and nutrition (Friedman, 1996; Franz & Erwin, 1990). Further, this reaction occurs even in vivo (Franz & Erwin, 1990; Thorpe & Baynes, 2003). In fact, the Maillard reaction follows a complex mechanism, and the final reaction products yield high molecular weight compounds called melanoidins. Recently,

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the researches on the Maillard reaction between chitosan and reducing sugars have been reported (Chung, Kuo, & Chen, 2005; Chung, Tsai, & Li, 2006; Tanaka, Huang, Chiu, Ishizaki, & Taguchi, 1993; Wang & Hon, 2003). However, the fundamental information is not yet provided enough. When considering the further application of chitosan to wood and wood components in the future, it is very important to clarify the basic characteristics of the Maillard reacted chitosan with reducing sugars that are related to wood components. In this study, cellulose model compounds such as glucose and cellobiose were used as reducing sugars. The characterization of the film obtained from the reaction between chitosan and the reducing sugars was investigated.

2. Experimental

2.1. Materials

Purified chitosan powder from a crab (*Chionoecetes japonicus*) was obtained from Kimica Corporation (Tokyo, Japan). The degree of deacetylation and molecular weight were 85.6% (colloid titration method) and 35,000 (viscosity method), respectively. D-Glucose and cellobiose were obtained from Sigma–Aldrich Japan Ltd. (Tokyo, Japan). All materials were vacuum-dried at 60 °C for 24 h. Acetic acid, N/400 potassium polyvinylsulfate, and 0.1% toluidine blue indicator solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Film preparation

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

2.3. 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.

2.4. Color measurement

The films were conditioned at 20 °C and RH65% for more than 48hr prior to measurement. The film was set on five white filter paper sheets. The film color was measured using a colorimeter (Gardner color-guide 45/0, Toyo Seiki Seisaku-sho Ltd., 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-degree. Three positions of each film were tested and the average value was used to evaluate the film color.

The CIELab color parameters (L^* , a^* , and 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.

2.5. FT-IR measurement

All infrared spectra of the films were obtained with an FT-IR620 spectrometer (JASCO) by using the KBr pellet method and were recorded by an average of 64 scans at a resolution of 4 cm^{-1} .

2.6. 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 5wt% acetic acid solution 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 adding 2–3 drops of 0.1% toluidine blue indicator, the filtrate was titrated against N/400 potassium polyvinylsulfate. The titration was carried out in triplicate, and average value was calculated. 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 change and FT-IR analysis were performed.

2.7. Tensile test

Tensile test was performed using ORIENTEC STA-1150. First, films were allowed to stand at 20 °C, RH

60% for more than a week, and rectangular specimens (40×8 mm) were cut out from the film using a paper cutter. About fifteen specimens were tested for each condition. The specimens fractured at near to grip were excluded, and the 10 specimens out of fifteen were adopted as the data. Initial grip separation and cross-head speed were 30 mm and 2 mm/min, respectively.

3. Results and discussion

3.1. Weight increase and color change

Fig. 1 shows the weight increase of each chitosan film to which the model compounds were added. Regardless of the compounds, the weight increased similarly up to 50wt% addition. In the case of 70wt% addition, the average weight increases of glucose and cellobiose were 157% and 207%, respectively. This indicated that each compound was remarkably incorporated into chitosan as the addition amount increased.

Fig. 2 shows the color change of each compound-added chitosan film. The ΔL^* value of glucose-added film decreased drastically with the addition amount. The value of cellobiose-added film also decreased continuously. The ΔE^* value of glucose-added film increased rapidly up to 50wt% addition. The value of cellobiose-added film also increased with the addition amount. In the case of the 50wt% and 70wt% additions, similar values were detected in both compounds. In fact, the compound-added chitosan films developed a dark brown color with increasing the addition amount, indicating that the Maillard reaction was proceeding.

3.2. Change of chemical structure

Fig. 3 shows the infrared spectral changes of glucose-added chitosan film. Regarding the infrared spectrum of pure chitosan, the assignments of several peaks have been already clarified. The characteristic absorption bands

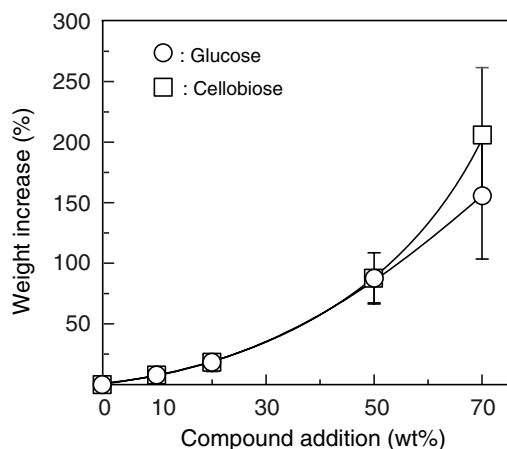


Fig. 1. Weight increases of the compound-added chitosan films.

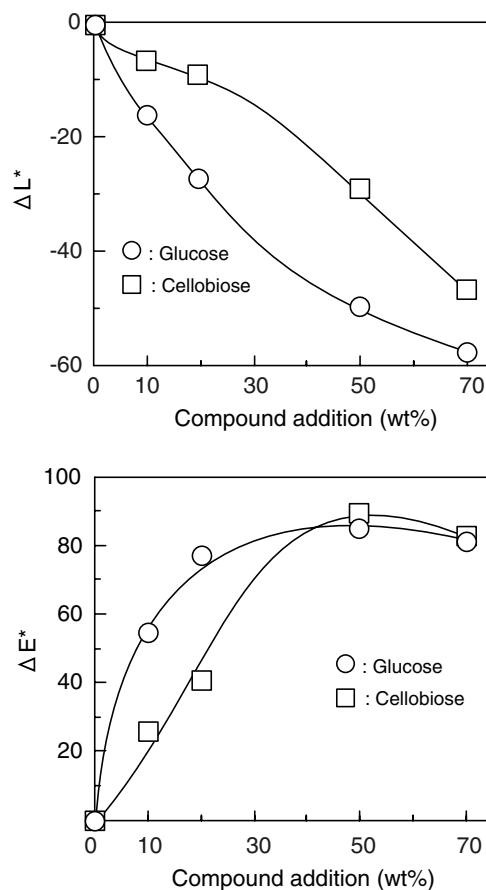


Fig. 2. Changes in brightness (ΔL^*) and color (ΔE^*) differences of the compound-added chitosan films.

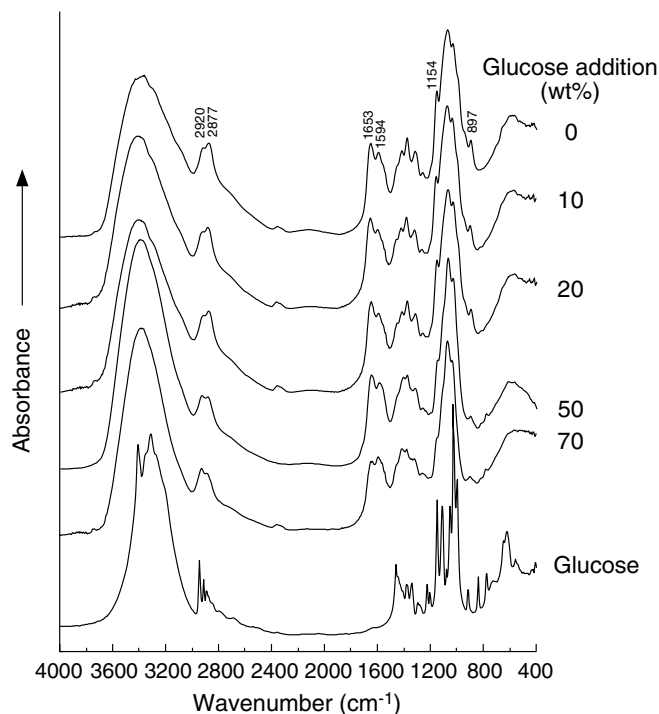


Fig. 3. Infrared spectra of glucose-added chitosan films.

between 4000 and 2500 cm^{-1} are 3480–3440, 3260–3270, and 2960–2878 cm^{-1} for the OH, NH and the CH stretching regions, respectively (Mucha & Miśkiewicz, 2000). The adsorption bands at around 1650 cm^{-1} , around 1560 cm^{-1} , and around 1310 cm^{-1} are assigned to amide I, amide II (Japanese Society for Chitin & Chitosan ed., 1995; Sannan, Kurita, Ogura, & Iwakura, 1978), and amid III (Sannan et al., 1978), respectively. In this study, the absorption peak at 1594 cm^{-1} that is attributed to the primary amino group was also observed. The adsorption bands at around 1157 and 897 cm^{-1} are assigned to the C–O stretching and the β -D-configuration (Japanese Society for Chitin & Chitosan ed., 1995; Hirano, 1978; Hirano, Matsuda, Miura, & Tanaka, 1979), respectively. When glucose was added to chitosan, the absorption peaks at around 1154 and 897 cm^{-1} gradually decreased. This suggested that the sugar unit of chitosan was cleaved with increasing glucose addition. In addition, slight changes were observed in amide I and II regions.

Fig. 4 shows the infrared spectral change of cellobiose-added chitosan film. Similar to glucose, the absorption bands at 1154 and 897 cm^{-1} gradually decreased, showing that the cleavage of sugar unit occurred. The changes in amide I and II regions were also observed. In particular, the peak at about 1560 cm^{-1} showed a sharp increase.

As amino groups were consumed during the Maillard reaction, the relative amount of free amino groups was observed in each film. The results are shown in Fig. 5. The free amino groups of glucose-added chitosan film decreased rapidly with increasing glucose addition. When glucose was added more than 20wt%, the value was less than 10%. This indicated that almost amino groups in

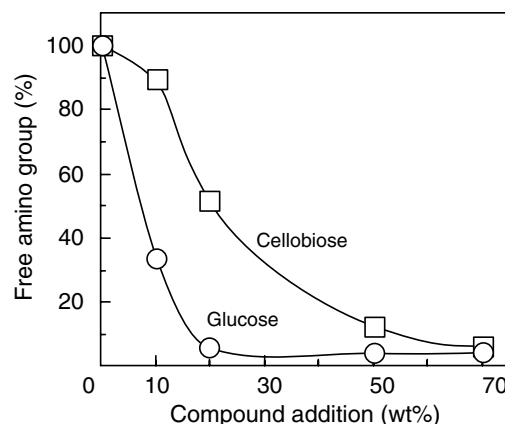


Fig. 5. Free amino group of the compound-added chitosan films.

the film were consumed by the Maillard reaction. In the case of cellobiose, the value decreased gradually with increasing cellobiose addition amount. Compared to the glucose addition, the marked differences were observed in a small addition amount. This could be due to the different reactivity based on the molecular weight and the chemical structure. It was clarified that Maillard reaction proceeded easily by the glucose addition of a small amount.

3.3. Insoluble matter

Fig. 6 shows the insoluble matter of each chitosan film in 5wt% acetic acid solution. In glucose-added chitosan, the insoluble matter increased rapidly and then decreased. The maximum average value recorded was 93% in 20wt% addition. The insoluble matter of the cellobiose-added chitosan increased gradually until 50wt% addition, and the maximum average value obtained was 64%. Generally, chitosan is soluble in a dilute acid to form the salt. However, it was demonstrated that chitosan changed to

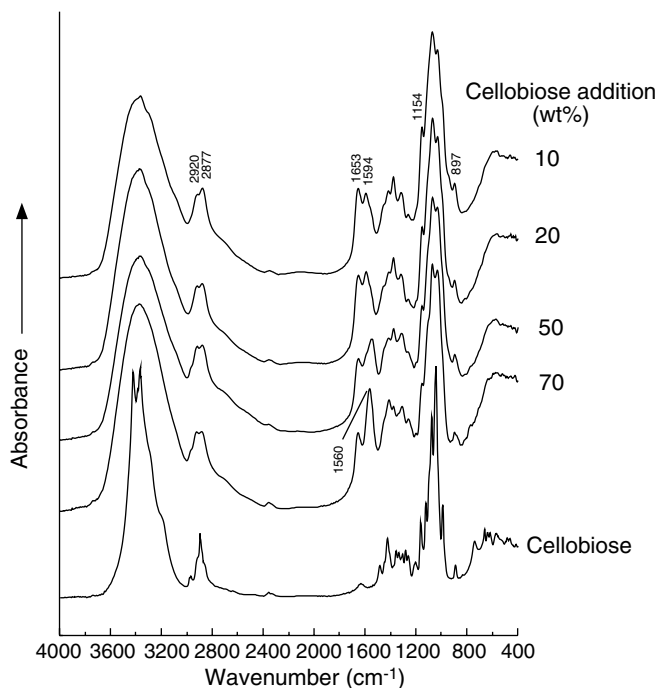


Fig. 4. Infrared spectra of cellobiose-added chitosan films.

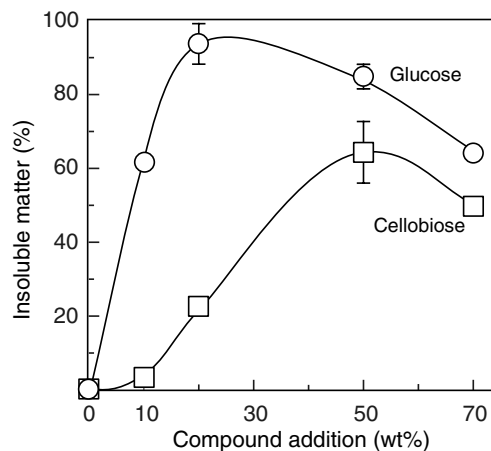


Fig. 6. Insoluble matter of the compound-added chitosan films in 5% acetic acid solution.

insoluble matter by adding glucose and cellobiose. Considering the result of Fig. 5, the increase of the insoluble matter was related to the decrease of free amino group. The decrease of the insoluble matter in higher addition amount would be due to some acetic acid-soluble substances formed by the Maillard reaction.

Figs. 7 and 8 show the infrared spectra of insoluble matters of glucose and cellobiose-added chitosan films, respectively. In the case of 10wt% glucose addition (Fig. 7), absorption bands at 1646 and 1567 cm^{-1} were clearly observed. As mentioned in Fig. 3, the adsorption bands at around 1650 cm^{-1} and around 1560 cm^{-1} were attributed to C=O (amide I) and N–H (amide II), respectively. However, considering the results of Figs. 5 and 6, the formation of Schiff base was expected. The absorption band at around 1650 cm^{-1} had been assigned to C=N linkage (Hirano et al., 1979; Kurita, Ishiguro, & Kitajima, 1988). The intensity of the band at 1567 cm^{-1} decreased with increasing glucose addition amount. This indicated that a lot of tertiary amines came to be formed instead of secondary amines. The absorption bands at 1153 and 900 cm^{-1} attributed to the sugar unit of chitosan also decreased gradually. The absorption bands at approximately 1716, 1460, and 778 cm^{-1} appeared as a small shoulder in the additions of 50% and 70%, respectively. It is known that the Maillard reaction is mainly divided into three stages (Martins, Van Jongen, & Boekel, 2001). Initial reaction between aldose sugar and an amino compound leads to the formation of the Amadori product via the Schiff base. In the subsequent reaction, the Amadori product is broken down into numerous products. When the pH value of the system is 7 or below, the main products

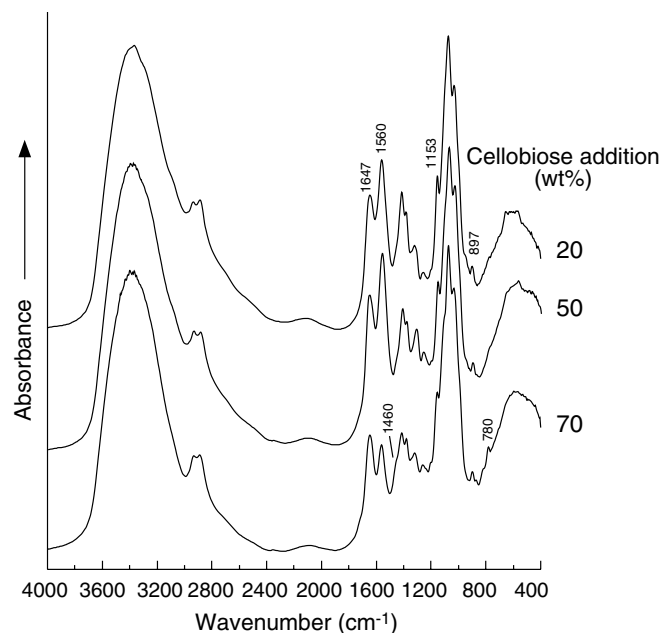


Fig. 8. Infrared spectra of insoluble matter in cellobiose-added chitosan films in 5% acetic acid solution.

formed are furfural and its derivative. In the final stage, the reaction products condense with amino compounds and the brown nitrogenous polymers and copolymers named melanoidins are finally formed. In this study, the reaction system was acid condition because 1wt% acetic acid was used. Therefore, the appearance of the absorption bands at around 1716 cm^{-1} would be the formation of carbonyl groups such as aldehydes and ketones. In addition, the absorption bands at around 1460 and 778 cm^{-1} seemed to be due to the formation of heterocyclic compounds.

In the case of 20wt% cellobiose addition (Fig. 8), the absorption band at 1647 cm^{-1} was identified clearly, indicating that the Schiff base was formed. The bands at 1560, 1153, and 897 cm^{-1} decreased with increasing cellobiose addition as with the glucose. The absorption bands at around 1460 and 780 cm^{-1} were observed as small shoulders in the 70wt% addition. However, the absorption band at around 1700 cm^{-1} was not observed even in the 70wt% addition.

Based on the above results, amino groups of chitosan were consumed remarkably with increasing an addition amount of glucose and cellobiose. As a result, the films changed to the insoluble matter to dilute acetic acid. However, when an enough amount was added to chitosan, the severe reaction proceeded with the cleavage in sugar unit of chitosan. In this case, some acetic acid-soluble substances were formed in the films.

3.4. Tensile strength

Fig. 9 shows the addition effects of glucose on the tensile properties of chitosan film. The tensile strength steadily increased and then decreased gradually. The maximum

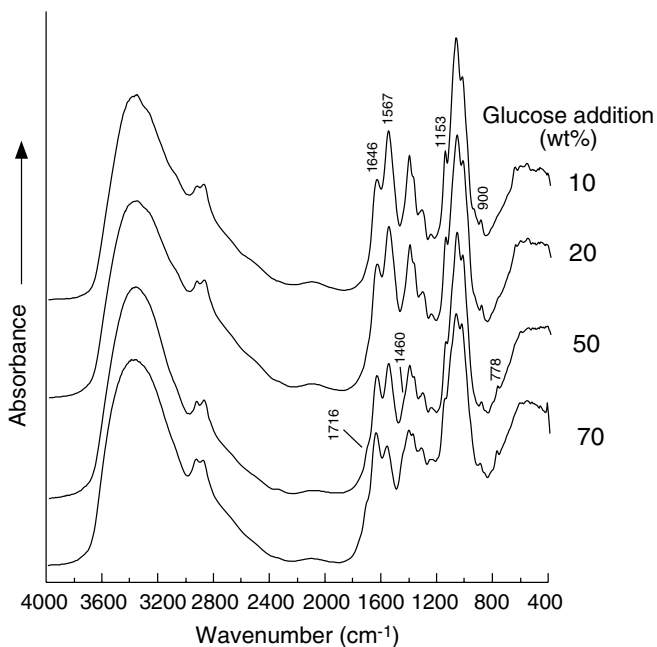


Fig. 7. Infrared spectra of insoluble matter in glucose-added chitosan films in 5% acetic acid solution.

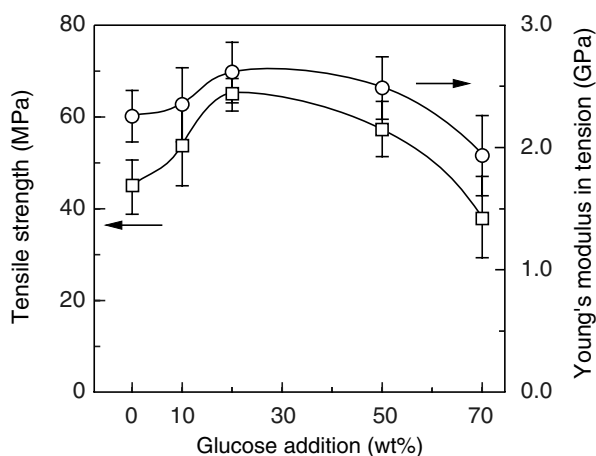


Fig. 9. Tensile properties of glucose-added chitosan films.

average value recorded was 64.8 MPa in 20wt% addition. Compared to pure chitosan film, the tensile strength improved about 45%. In Young's modulus, the maximum value was also recorded in 20wt% addition, which was 2.6 GPa. Fig. 10 shows the effect of cellobiose on the tensile properties. As with glucose addition, the tensile strength improved about 30% in 20wt% addition. However, when cellobiose was added further more, the film strength lowered drastically. In Young's modulus, similar behavior was observed. Considering the results of FT-IR, the sugar units of chitosan existed until 20wt% addition of the compounds. 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 decreased due to the cleavage in sugar unit and the formation of heterocyclic compounds. For these reasons, tensile properties lowered. Therefore, it was made clear that the tensile properties of chitosan were easily improved by a small amount addition of the compounds.

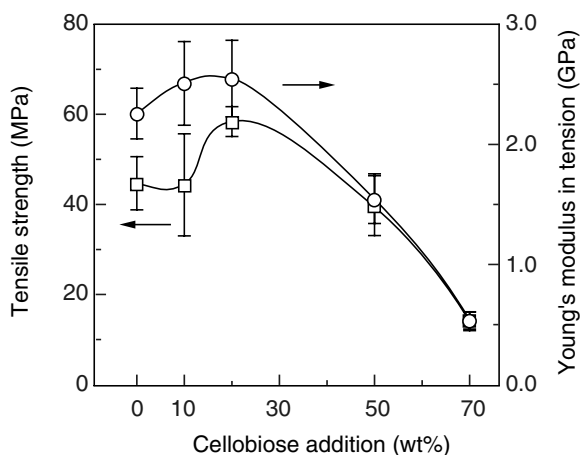


Fig. 10. Tensile properties of cellobiose-added chitosan films.

4. Conclusions

When the cellulose model compounds were added to chitosan, the weight of the film obtained increased markedly with increasing amount of the compounds. In addition, the color difference of the film became larger and the free amino group of the chitosan decreased rapidly. The insoluble matter of the film to 5wt% acetic acid solution increased and then decreased gradually. In particular, the glucose-added film was almost insoluble at 20wt% addition. Based on the results of FT-IR spectra, the consumption of amino groups such as the formation of the Schiff base were observed in a small addition of the compounds. The Maillard reaction occurred significantly with the formation such as heterocyclic compounds and the cleavage in sugar unit of chitosan as the addition amount of the compounds increased. The tensile properties of the film improved until 20wt% addition of the compounds, and then lowered. The reactivity of glucose to chitosan was superior to that of cellobiose. A 20wt% addition of glucose was especially effective to improve the properties of chitosan film.

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