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SHORT COMMUNICATION

Preparation of tannin gel by enzyme-mimetic reaction of condensed tannin without use of crosslinking agent

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Abstract Preparation of tannin gels was attempted by means of enzyme-mimetic reaction of condensed tannin without use of crosslinking agent. The gelation of condensed tannin was performed in the presence of H_2O_2 catalyzed by hematin of a ferriprotoporphyrin complex in water. The compressive measurements of the gels obtained by the 30–50 wt% concentrations of the tannin using 0.20–0.50 ml of 30 *w*/*v*% H_2O_2 aq. were examined. There is a tendency for the compressive properties of the gels obtained from the higher concentrations of the tannin and the larger amounts of H_2O_2 to be superior. The obtained gel was characterized by means of IR spectroscopy. Moreover, the obtained tannin gel was compatibilized with natural rubber to form a composite material.

Keywords Condensed tannin · Enzyme-mimetic · Hematin · Crosslinking · Gel

Introduction

Condensed tannins (also called polymeric proanthocyanidins) are natural polyphenolic substances found in abundance in many woody plants [1]. Certain condensed tannins are produced commercially from woods and barks and they have been used as a raw material for the production of tannin-formaldehyde wood adhesives since the 1970s [2, 3]. Structurally, condensed tannins are flavanoid polymers [1], which vary based on biosynthetic source. They can be further subclassified in a number of structural groups based on the hydroxylation pattern of the repeating flavanoid monomer units. The representative chemical structure of procyanidin, which has been the most common in nature, is shown in Fig. 1. The alcoholic hydroxy groups are sometimes replaced to gallic acid esters.

Because of the polyphenolic structure of condensed tannins as shown in Fig. 1, they can be crosslinked by formaldehyde to produce the corresponding gels [4–7]. Based on the viewpoints of environmentally aspects, however, efforts should be made to reduce formaldehyde emissions by the replacement with other crosslinking procedures. As preparation of the tannin gels without use of such the chemical crosslinking agent, i.e., formaldehyde, for example gelation of condensed tannin in the presence of sodium borate/calcium chloride was reported [8]. Since an oxidoreductase enzyme, horseradish peroxidase (HRP), acting on H_2O_2 as oxidant has been widely used as the catalyst for the oxidative reaction of phenolic compounds, such as the oxidative polymerization of phenols and (iso) flavonoids [9–12], enzymatic crosslinking reaction of condensed tannin using HRP is expected to proceed, forming the corresponding crosslinked materials without use of formaldehyde. However, it is well known that condensed tannin has a high affinity with proteins to produce the complexes [13], indicating that the tannins may inhibit the enzymatic reaction.

The HRP enzyme is a ferric heme species, and reacts with H_2O_2 to form a catalytically active reaction interme-

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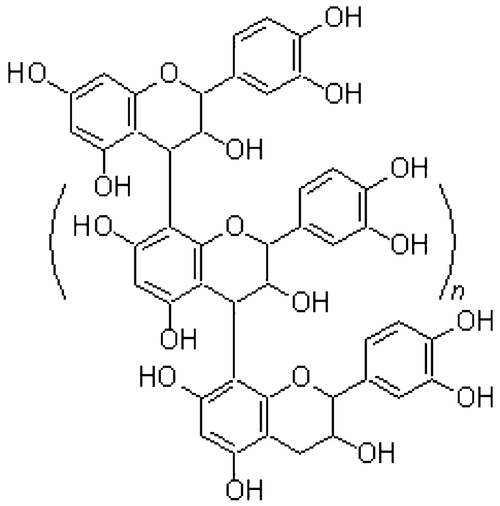

Fig. 1 Representative structure of condensed tannin

diate, which induces the oxidative reaction of phenols. The free heme, a ferriprotoporphyrin, is unstable, and rapidly oxidized to hematin (Fig. 2). Hematin has been well known as a premising alternative catalyst to replace HRP for the enzyme-mimetic oxidative reaction of phenols and other substrates [14, 15]. This compound has been inspired us to be employed as the efficient catalyst for crosslinking reaction of condensed tannin because hematin does not contain proteins, that is different from HRP. In this paper, we report preparation of tannin gel by the enzyme-mimetic crosslinking reaction of condensed tannin catalyzed by hematin. The compressive stress-strain measurements of the obtained gels were also carried out. As the preliminary application of the tannin gel, furthermore, a composite composed of the crosslinked tannin and natural rubber was prepared.

Experimental

Materials and methods

Condensed tannin (a proanthocyanidin polymer) was provided in freeze-dried powder by Maruzen Pharmaceuticals, Japan. The natural polymer was purified from Kakishibu (a fermented persimmon juice) using HP column chromatography. The purity was more than 95%, which expressed as mg flavonoid per dry weight of tannin. Other reagents and solvents were used as received. Thermal gravimetrical analysis (TGA) was performed on a SII TG/DTA6200 at a heating rate of 10°C/min.

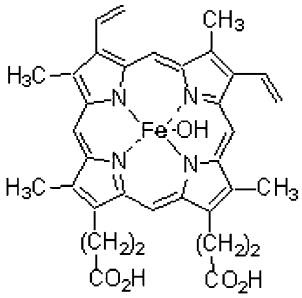

Fig. 2 Structure of hematin

Preparation of tannin gels

Condensed tannin (5.0 g, 17.4 mmol based on the representative structure in Fig. 1) was dissolved in 10 ml of water. After hematin (0.010 g, 0.016 mmol) was added to the solution, 30 *w*/*v*% H_2O_2 aq. (0.20 ml, 1.76 mmol) was added dropwise with stirring, and the resulting viscous solution was vigorously mixed for 1 h. The mixture was kept further for 16 h to give the gel.

Compressive stress-strain measurements

The samples for the measurements were prepared by cutting the gel to a column style (diameter; ca. 25 mm, height; ca. 10 mm). The measurements were carried out with the compressive rate of 1.0 mm/min using a tensile tester (Little Senstar LSC-1/30, Tokyo Testing Machine).

Preparation of composite composed of crosslinked tannin and natural rubber

Condensed tannin (2.0 g, 6.94 mmol) was dissolved in 6.1 ml of water. After hematin (0.0039 g, 6.16 μmol) and 30 *w*/*v*% H_2O_2 aq. (0.80 ml, 7.06 mmol) was successively added to the solution, rubber latex (60 wt%, Regitex, Atsugi, Japan) was added with stirring and the resulting viscous solution was vigorously mixed for 1 h. The mixture was transferred to a tray (100×70×13 mm) and kept further for 16 h at room temperature to give the relatively dried material. The material was put into a heat device (100×100×0.5 mm) and hot-pressed at 100°C and 20 MPa for 1 h and subsequently at 120°C and 20 MPa for 1 h to give the composite.

Results and discussion

Condensed tannin used in this study could be dissolved at most in the concentrations of ca. 50–60 wt% in water. Therefore, an attempt for preparation of tannin gel was made under some conditions in the range of 10–50 wt% concentrations of the tannin in water. When gelation was performed under the following conditions, i.e., in the presence of hematin (0.020 mmol) using 30 *w*/*v*% of H_2O_2 aq. (0.20–0.50 ml) in the 30–50 wt% concentrations

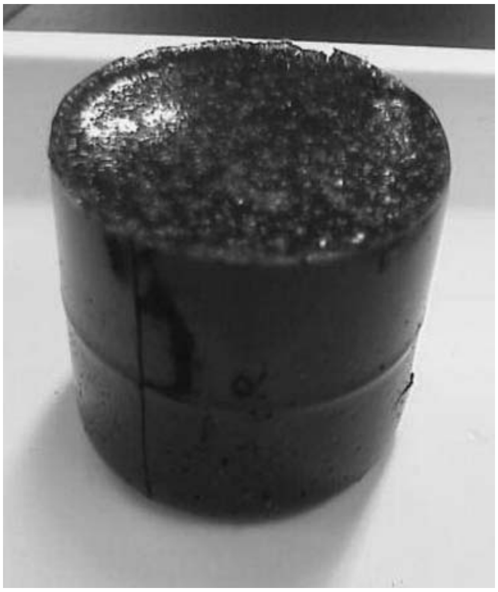

Fig. 3 Photograph of obtained tannin gel

of the tannin in water (10 ml), the gels having relatively elastic and good mechanical properties were obtained, which had been supplied for the compressive measurements. The representative appearance of the obtained gel is shown in Fig. 3.

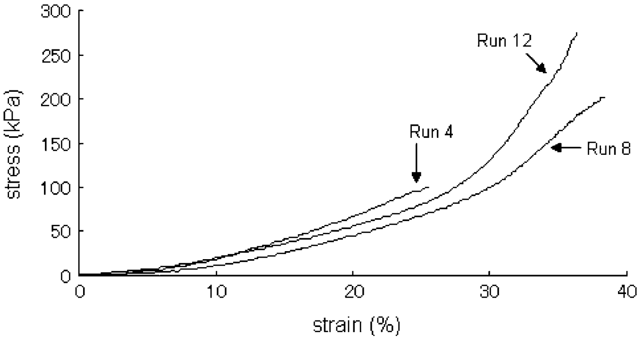

Fig. 4 Stress-strain curves of representative gels obtained by the 30, 40, and 50 wt% concentrations of the tannin using the same amount of H_2O_2 (4.41 mmol; run 4, 8, and 12)

Table 1 shows the conditions for preparation of gels and their properties. The water contents were estimated by the weight differences of the dried materials from the gels, which had been relatively close to those of water amounts used for the gel preparation (76.9, 71.4, and 66.7% for 30, 40 and 50 wt% concentrations, respectively). In each concentration of the tannin, the gels relatively sustain the higher stresses when the larger amounts of H_2O_2 are used, probably caused by more frequent crosslinking reactions. As shown in Fig. 4, the fracture stresses increase with increasing the concentrations of the tannin when the conditions using the same amount of H_2O_2 were compared. The fracture strains obtained by the concentration of 40 wt% are higher than those formed by the concentration of 30 wt %. However, the elasticities of the gels from the concentration of 50 wt% are comparable to those formed by the concentration of 40 wt%. In conclusion, the compressive property of the gel obtained by the concentration of 50 wt% using a larger amount of H_2O_2 (run 12, fracture stress; 275.8 kPa, fracture strain; 36.4%) is more superior to that from other concentrations. The values of the fracture stresses and strains of the gels are listed in Table 1, which are in the ranges of 67.3–275.8 kPa and 25.5–38.5%, respectively.

Figure 5 shows the IR spectra of the condensed tannin (a) and the dried gel (run 12; b), respectively. Both the spectra exhibit the absorptions at around 1,700 cm^{-1} due to the C=O groups, indicating partial replacement of alcoholic groups to the gallic acid esters. In the spectrum of the dried gel (Fig. 5b), the broad absorption centered at 3,448 cm^{-1} due to the OH linkage of the phenolic and hydroxyl groups and the absorptions at around 1,535–1,620 cm^{-1} ascribed to the aromatic C=C groups are observed. Furthermore, the spectroscopic pattern is quite similar as that of the original condensed tannin (Fig. 5a). On the basis of the above data, we assume that the structure of the gel is mainly composed of the C–C crosslinking linkages at aromatic rings. The formation of the similar C–C linkages was reported in the previous study on the HRP-catalyzed polymerization of catechin and related compounds [12]. However, the crosslinking densities in the gels are not yet estimated because of the complicated structure of the original condensed tannin.

Table 1 Results of preparation of tannin gels in the presence of H_2O_2 catalyzed by hematin[a]

Run	Tannin g (mmol[b])	30 *w*/*v*% H_2O_2 ml (mmol)	Water content[c] %	Fracture stress[d] kPa	Fracture strain[d] %
1	3 (10.4)	0.2 (1.76)	75.5	67.3	32.2
2	3 (10.4)	0.3 (2.65)	76.6	83.9	28.3
3	3 (10.4)	0.4 (3.53)	71.1	85.2	26.1
4	3 (10.4)	0.5 (4.41)	73.2	100.1	25.5
5	4 (13.9)	0.2 (1.76)	69.6	103.9	35.1
6	4 (13.9)	0.3 (2.65)	69.5	110.5	36.0
7	4 (13.9)	0.4 (3.53)	67.8	180.5	34.5
8	4 (13.9)	0.5 (4.41)	66.5	202.4	38.5
9	5 (17.4)	0.2 (1.76)	63.9	148.4	35.3
10	5 (17.4)	0.3 (2.65)	66.4	185.5	37.1
11	5 (17.4)	0.4 (3.53)	60.9	221.9	36.1
12	5 (17.4)	0.5 (4.41)	61.3	275.8	36.4

[a] Reaction was carried out using hematin (0.010 g, 0.016 mmol) in water (10 ml) at room temperature.

[b] Based on a representative structure of condensed tannin as shown in Fig. 1.

[c] Water content was estimated by the weight difference of the dried material from the gel.

[d] Fracture stress and strain were determined by compressive stress-strain measurement using a tensile-compressive tester.

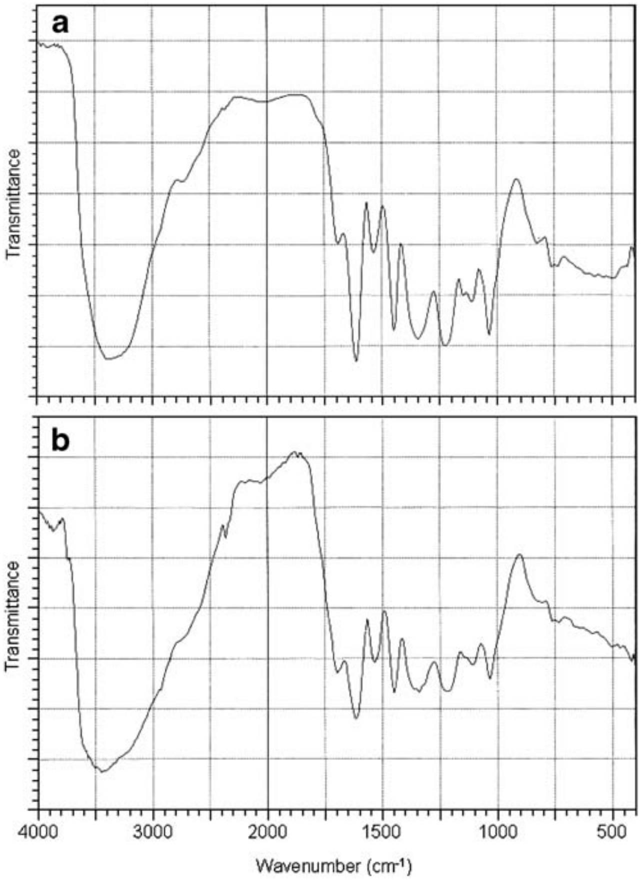

Fig. 5 IR spectra of condensed tannin (**a**) and dried gel (run 12; **b**).

As one of the applications using the tannin gel, its compatibilization with other natural polymers has been considered to obtain the composite materials composed of natural resources. For example, a preliminary attempt of the compatibilization with natural rubber was made to produce the corresponding composite. The preparation of the composite was performed by the above-mentioned gelation method of the condensed tannin in the presence of rubber latex. The obtained viscous mixture was dried and further hot-pressed to give the composite. As shown in Fig. 6, the obtained material [tannin: rubber=1:1 *w*/*w*)] showed the relatively flexible property. Figure 7a shows the TGA curve of the composite material in comparison with that of natural rubber and original tannin (Fig. 7b and c, respectively). The weight loss profile of the composite is relatively different from other two profiles, assuming a good compatibilization of the two components in the composite. The detailed studies on preparative conditions, characterizations, and properties of the composite materials are now in progress.

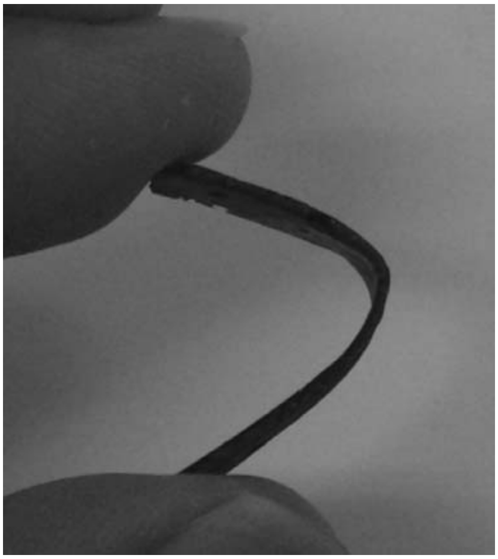

Fig. 6 Photograph of composite composed of crosslinked tannin and natural rubber [1:1 (*w*/*w*)]

Conclusion

Tannin gels were prepared by hematin-catalyzed enzyme-mimetic reaction of condensed tannin without use of the crosslinking agent. The gelation was performed in the 30–50 wt% concentrations of the tannin in the presence of H_2O_2 catalyzed by hematin in water. The obtained gels showed relatively good mechanical properties, which were estimated by the compressive measurements. The tannin gels in the present study, which are formed by the enzyme-mimetic crosslinking reaction, can be considered as environmentally benign materials. For example, its compatibilization with other natural polymers is possible for preparation of the composite materials composed of natural resources. As described above, we have already demonstrated preliminary preparation of the composite composed of the crosslinked tannin and natural rubber. The detailed studies on applications of the gels, such as one component of the composites shown as above, as well as the materials with deodorizing and sterilizing effects, are under way in our laboratory.

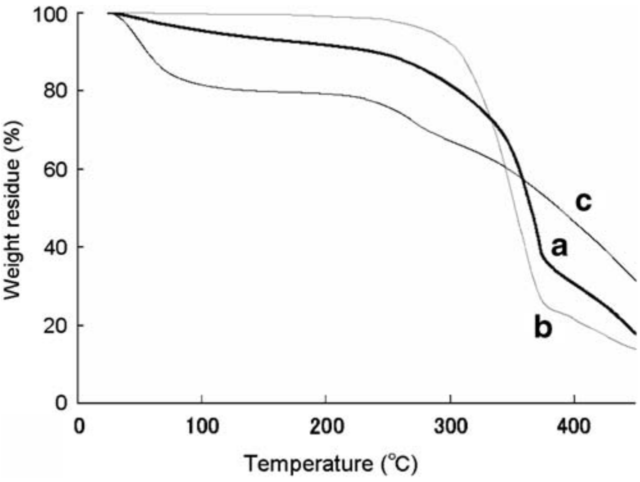

Fig. 7 TGA profiles of composite (**a**), natural rubber (**b**), and condensed tannin (**c**)

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