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Note

Thermoreversible gel formation of water soluble extract from *Sparassis crispa*

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Rheological properties of the extract from *Sparassis crispa* (*S. crispa*) were investigated. The extract was prepared from water soluble fraction of the pureed *S. crispa*. The frequency dependence of the storage shear modulus *G*' and the loss shear modulus *G*" changed from sol to gel type behavior on cooling, indicating that gel formation occurred. The gelation temperature was around 5 ºC and the gel showed thermoreversible. The main component of the extract was *β*-glucans, over 80 % of the dry weight of the extract. From these results a novel texture modifier consisting of bioactive *β*-glucans produced from the extract of *S. crispa* was proposed.

Keywords: thermoreversible gel, *β*-glucan, *Sparassis crispa*, mushroom

Sparassis crispa (*S. crispa*) is an edible mushroom cultivated at several laboratories and companies in Japan. The fruiting bodies of *S. crispa* show preferable and characteristic texture when cooked appropriately. The characteristic texture of *S. crispa* might relate to specific composition of polysaccharides,

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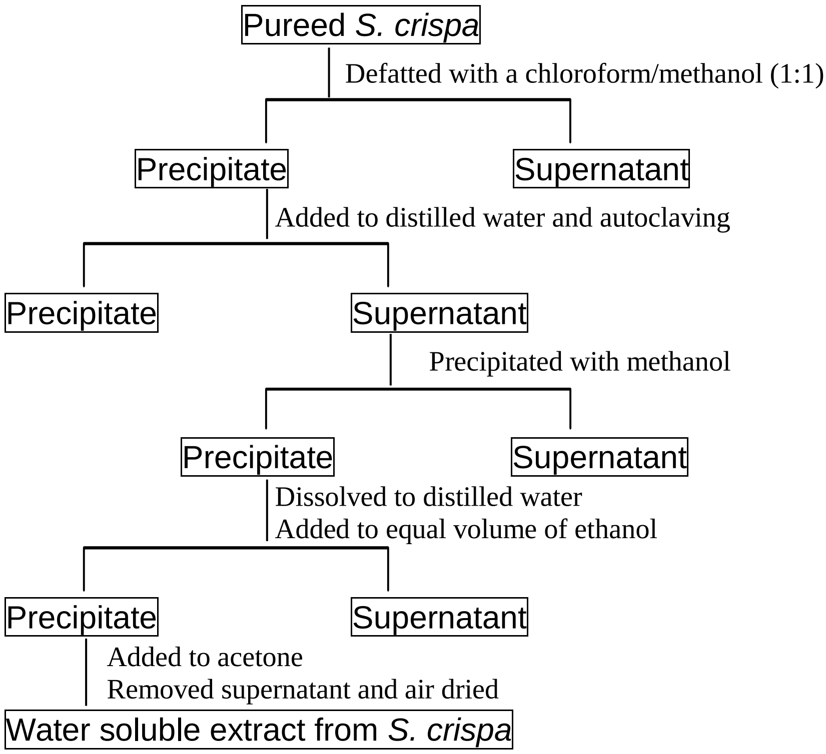
where *S. crispa* is reported to contain β-glucan abundantly (about 40 % of the dry weight of the fruiting bodies) [1]. The mushroom β-glucans typically are composed of a (1,3)-β-glucan main chain with short side chains linked (1,6)-β- to main chain [2]. The primary structure of β-glucan of *S. crispa* is reported to be (1,3)-β-glucan main chain with single (1,6)-β-glucosyl side branching units every three residues [1].

Fungal β-glucan has been investigated mainly from the viewpoint of health benefits like antitumor activity. Biological activities of β-glucan of *S. crispa* has been investigated extensively [3-5] but food application of the β-glucan of *S. crispa* has not been investigated so much. Some β-glucans such as curdlan ((1,3)-β-glucan) and cereal-based β-glucans ((1,3/1,4)-β-glucan) are known to form a gel [6]. Preliminary study showed that when the fruiting bodies of *S. crispa* were turned into puree, the viscosity of the puree increased steeply on lowering the temperature. The puree became gel-like at lower temperature and then became viscous liquid again by raising temperature; thermoreversible gel formation seemed to occur. Themoreversible gel, especially cold-setting gel, is used in a wide range of food products. Gelatin and agarose are often used as cold-setting gelling agents and their gel properties do not always satisfy food manufacturer needs. Because of limited polymers having a gelling ability, novel gelling agents are required for the alternative of gelatin and agarose. In the present study we investigated whether gelling component can be obtained from *S. crispa*.

Material and Methods

Preparation of extract from S. crispa - Fruiting bodies of *S. crispa* were cultured at Laboratory of Yamatokingaku, Nara. The fruiting bodies of *S. crispa* were added to water and were pureed with a blender. Water soluble extract from *S. crispa* was prepared as follows; the pureed *S. crispa* was defatted with a mixed solvent chloroform/methanol (1:1) at room temperature overnight. The resulting residue was added to distilled water and heated by autoclaving (121 ºC for 20 min). After centrifugation (25 ºC, 10000 rpm for 10 min) supernatant was collected and precipitated with a large volume of methanol. The precipitate was redissolved to distilled water and then equal volume of ethanol was added. The resultant precipitate was added to acetone and mixed by a vortex mixer and then centrifuged (25 ºC, 10000 rpm for 10 min). Supernatant was removed and the precipitate was air dried to use as the water soluble extract from *S. crispa*. For the sample of rheological measurement, the extract was dispersed in distilled water to be 1.5 wt% and stirred overnight at room temperature.



Scheme 1 Preparation of the water soluble extract from the pureed *S. crispa*.

Rheological measurement - The storage shear modulus *G*' and the loss shear modulus *G*'' were measured by a Rheostress 600 (Haake, Germany). The sample was heated to 90 °C and then poured onto a parallel plate geometry (plate diameter: 35 mm) at 40 °C. The periphery of the sample was immediately covered with silicone oil to prevent the evaporation of water. Stress dependences of *G*' and *G*'' were examined to determine a linear viscoelastic regime. Within this regime, *G*' and *G*'' were measured at various temperatures and frequencies.

β-Glucan content analysis - β-Glucan content was estimated by the Glucan Enzymatic Method (GEM) proposed by Danielson et al [7].

Results and Discussion

The frequency dependences of the storage shear modulus *G*' and the loss shear modulus *G*'' for 1.5 wt% extract from *S. crispa* are shown in Fig. 1. At 38 °C, *G*' was larger than *G*'' with increasing frequency at all tested frequencies (Fig. 1 (a)) . This behavior has previously been called as a “weak gel” type behavior: *G*' is larger than *G*'' in a wide range of frequencies and mechanical loss tangent tanδ (=*G*''/ *G*') is always larger than 0.1. As mentioned previously [8], this terminology is not the most accurate description because a “weak gel” is essentially a liquid and not a gel. A typical sample showing the “weak gel” type behavior is a xanthan solution that is used in sol like foods, e.g., sauce and dressing. The “weak gel” type behavior indicates that the sample is still a viscoelastic liquid. In Fig. 1 (b), at 2 °C *G*' and *G*" were almost independent of frequencies and tanδ was lower than 0.1, which showed a typical gel-like behavior. This indicates that sol-to-gel transition occurs. Fig. 2 shows temperature dependences of *G*' and *G*" at 1 rad/s and indicated the steep increase of *G*' around 5 °C, suggesting that gelation occurs on cooling below 5°C. On subsequent heating, *G*' decreased with increasing temperature and the thermal hysteresis disappeared around 30 °C (Fig 2), suggesting that the gel melting was completed up to 30 °C.

It has been reported that the water soluble extract prepared by the method of the present study contains β-glucan as a main component [9]. The

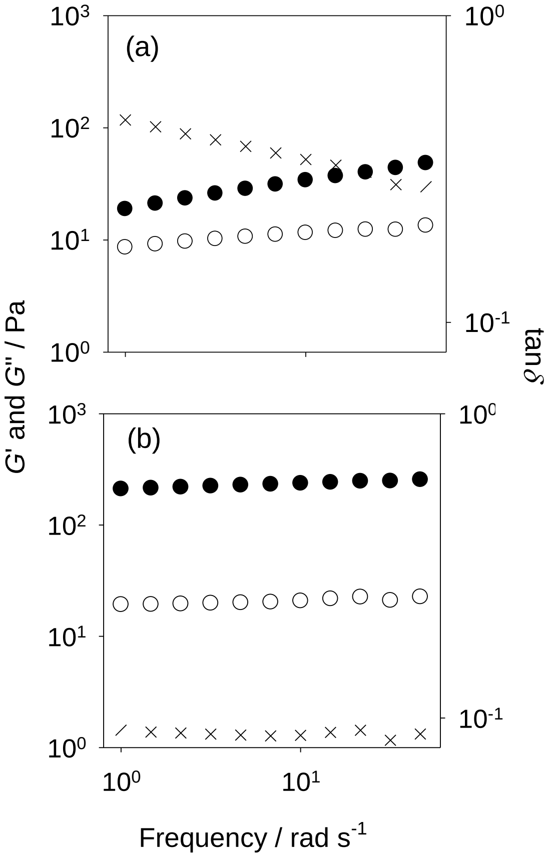


Figure 1. Frequency dependence of the storage shear modulus *G*' (●), the loss shear modulus *G*" (○) and mechanical loss tangent tanδ (×) for 1.5 wt% extract from *S. crispa* at (a) 38 ºC and (b) 2 ºC. Stress; 1 Pa.

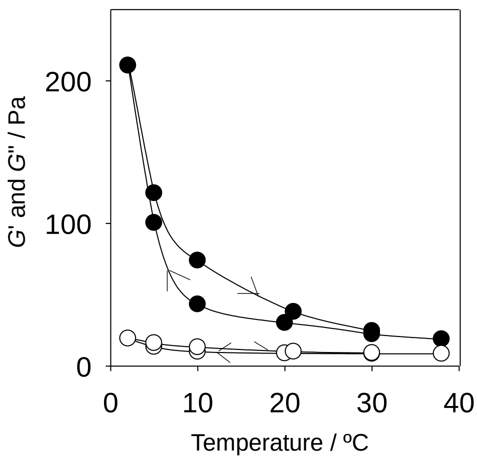


Figure 2. Temperature dependence of *G*' (●) and *G*" (○) at 1 rad/s on cooling and subsequent heating for 1.5 wt% extract from *S. crispa*. Stress; 1 Pa. The sample was kept at least 20 min at each temperature.

β-glucan content of the extract of the present study was examined by GEM assay and was estimated to be about 82 %. The β-glucan of *S. crispa* is reported to be (1,3)(1,6)-β-glucan [1] and if the (1,3)(1,6)-β-glucan formed a gel, it would be a novel example of thermoreversible gels. Some (1,3)(1,6)-β-glucans have been reported to have gelling ability [10] but the behavior of these glucans was of a "weak gel" type.

Polydispersity, molecular weight and branching ratio of the β-glucan would affect the gel properties. Contents of components other than the β-glucan would also affect gelation and gel properties. The factors influencing gelation and gel properties should be explored in future studies.

Figure 3. 1.5 wt% gel of *S. crispa*.

The gel in the present study may be transparent (Fig.3). Other properties related to the texture are now under investigation. A low gelling temperature and transparency of the gel are characteristics similar to gelatin gel, that produces a possibility of using the extract from *S. crispa* as an alternative of gelatin. β-Glucan of *S. crispa* has been focused on the role of physiological benefits like antitumor activity. The present study revealed that the extract containing β-glucan of *S. crispa* can be used as a gelling agent. It is interesting if the antitumor activity of *S. crispa* is related to its gelling ability although the relationship between the antitumor activity and gelling ability of the β-glucan has not been reported yet. Even if there is no relationship between them, the extract of *S. crispa* is valuable as just supplement of a bioactive β-glucan as well as just gelling agent for food application.

Conclusions

The extract from *S. crispa* could form a transparent, cold-setting, and thermoreversible gel. The gelation temperature was around 5 °C and gel melting was completed up to 30 °C. The main component of the extract was β-glucan, that was previously reported to show health beneficial activity. Thus the extract obtained in the present study might be useful as a novel gelling agent as well as a supplement of a bioactive β-glucan.

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