Determination of Acidic Component of Konjac Mannan

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Konjac mannan (KM), a glucomannan isolated from the tuber of *Amorphophallus konjac* C. Koch, is soluble in water, and the resultant sol is readily gelatinized on treating with alkali. The mechanism of gelation, however, has not been elucidated so explicitly. In the previous paper, it was demonstrated that no gelation sets in until the elimination of a low-molecular-weight acidic component from KM is brought about by alkali. The component, like acetyl groups of other polysaccharides, seems to play an important role in the rheological behavior of KM.

The determination of acidic component of KM was attempted by Torikata, who suggested the presence of acetate, uronate, and phosphate. However, it is questionable whether they are the constituents of KM, as no purification of KM was made at that time.

The present study was undertaken to determine the acidic component by means of chromatography, and analyses of saponifiable and volatile acids.

Tubers of *A. konjac* were collected at Yuki-cho, Hiroshima prefecture, in autumn 1974. According to the method described previously, the tubers were made into konjac flour, and purified KM (native KM) was prepared from the flour.

About 10 g of native KM was dissolved in 2 liters of water. To this sol was added 50 ml of 4% Na₂CO₃ with vigorous agitation for 1 min, and the mixture was gelatinized on standing for 1 hr at 40°C. The resultant gel was made into a slurry with 2 liters of methanol by use of a home mixer. After being allowed to stand for a while, the slurry was filtered through a glass filter by suction. The treatment of the filter cake with 50 methanol followed by filtration was repeated several times until the filtrate was no longer alkaline to litmus. The filter cake thus obtained was then peptized with a 20-fold excess of 2 M KSCN and purified in the same manner as applied to the native KM. The yield of the product (gelatinized KM) based on the native KM was about 95%. The methanolic filtrates were combined and concentrated to 50 ml at temperature below 30°C. A small portion of the solution was freed of CO₃²⁻ by passage through a Dowex 50w-x column (H⁺ form, 50~100 mesh, 2×20 cm), which was then washed several times with water. The combined effluent (50 ml) was neutralized with dil. NaOH and concentrated to 10 ml at 30°C under reduced pressure. The concentrate was subjected to a chromatographic analysis of saponified acid and a determination of volatile acid.

The saponification of native and gelatinized KM were carried out under the conditions mentioned previously. Apparent content of saponifiable acid was determined as 0.34 mmol/g (mean of 0.34, 0.34, and 0.35) and 0.01 mmol/g (mean of 0.01, 0.01, and 0.01) for native and gelatinized KM, respectively. In determination of true content of saponifiable acid, the amount of alkali consumed by gelatinized KM was subtracted from that of native KM as a blank, because no carboxyl or carbonyl group is present in gelatinized KM. Thus, the content of the component was found to be 0.33 mmol/g.

A qualitative analysis of saponified acid in the concentrated was carried out by a modification of the method of Ikawa et al. A liquid chromatograph (Nihon Denshi Kogyo Co., assembled from JLC-3, P-2, and JLC-A with a recorder) was used under the following conditions: column, Dowex 1×8 (200~400 mesh, 0.8×50 cm); column temperature, 35°C; mobile phase, 0.1 N HCl, flow rate, 0.5 ml/min. The chromatogram is shown in Fig. 1.
phase, 1 N HCl-NaCl buffer solution (pH 1.0); flow rate, 0.61 ml/min; detector, calorimetric detector; sensitivity, $3 \times 10^{-3}$ at 35°C; chart speed, 6.0 cm/hr; sample size, 1 ml. A typical chromatogram was given in Fig. 1. Only one signal with a retention time of 83 min was detected as an acidic component. No additional signal was obtained even after the development was continued for 300 min or over. The retention time of the component is very close to those of formate and acetate as shown in Table I. The peaks of formate, however, resolved slightly from those of the sample when authentic formate was added to the sample as an internal standard. Therefore, the component is chromatographically identified as acetate.

**Table I. Retention Times of Authentic Acids on Liquid Chromatography**

<table>
<thead>
<tr>
<th>Acid</th>
<th>$t_R$ (min)</th>
<th>Acid</th>
<th>$t_R$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formic</td>
<td>88</td>
<td>Lactic</td>
<td>72</td>
</tr>
<tr>
<td>Acetic</td>
<td>83</td>
<td>Pyruvic</td>
<td>94</td>
</tr>
<tr>
<td>Propionic</td>
<td>130</td>
<td>Glycollic</td>
<td>71</td>
</tr>
<tr>
<td>Butyric</td>
<td>240</td>
<td>Levulinic</td>
<td>103</td>
</tr>
<tr>
<td>Oxalic</td>
<td>212</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Volatile acid in the concentrate was determined by a modification of the Pregl–Sortys method. A 50-ml round bottomed flask attached with a capillary, a 10-ml separatory funnel, and a 30-cm condenser was employed as a distilling flask. The opposite end of the condenser was fitted with a 50-ml receiver through an adapter which was shaped so as to facilitate the entrapping of the distillate and connecting to a vacuum pump. Ten ml of the sample and 10 ml of 0.1 N NaOH were placed in the distilling flask and the receiver, respectively. The sample added of 4 ml of 20% p-toluenesulfonic acid was distilled to dryness at 100°C under a pressure of 25 mm-Hg in N2 atmosphere. The solution collected in the receiver was neutralized with 0.1 N HCl, acidified slightly with 0.01 N HCl, warmed on a boiling water bath for 20 min, and titrated with 0.01 N NaOH by using phenolphthalein as an indicator. The content of volatile acid in KM was thus found to be 0.34 mmol/g (mean of 0.33 and 0.34). The value is in fair agreement with that of saponifiable acid.

On the basis of the findings mentioned above, it can be concluded that the acidic component of KM is acetate or acetyl group only, and the molar ratio of this component to hexose residue in KM is estimated to be 1:19 if KM is represented by a molecular formula of $(\text{CH}_3\text{CO})_m(\text{C}_6\text{H}_{10}\text{O}_5)_n$.

The result is significantly different from that obtained by Torikata not only in the composition of acidic component but also in the content of acetyl group (acetyl group: hexose residue=1:9). The difference in the content of acetyl group is probably due to the purity of sample used and to the correction for acid content determined by saponification. At any rate, the content of acetyl group in KM is small as compared with those of other polysaccharides. Therefore, the role of this group in development of rheological properties of KM may be somewhat specific. Detailed investigation about this problem is in progress.

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**REFERENCES**


